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DOES USE OF

TETRACYCLINES AMONG VETERANS INCREASE THEIR RISK FOR

MELANOMA?

by

NANCY K. FAGAN

An Abstract

of a dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
Department of Epidemiology and Biostatistics
College of Public Health
University of South Florida

August 2001

Major Professor: Thomas J. Mason, Ph.D.

Studies have shown that people who have experienced episodes of acute exposure to ultraviolet radiation, resulting in severe sunburns, have an increased risk of melanoma skin cancers. Sunburns are photosensitive reactions to sunlight. Ingested chemicals, such as tetracycline antibiotics, can also cause photosensitive reactions. The tetracycline molecules in the skin absorb normally harmless doses of visible and UV light, clinically causing an acute inflammatory response (erythema) or "exaggerated sunburn".

Although photosensitivity is a well-recognized adverse event of the tetracycline class of antibiotics (studies reporting 3 to 90% of users experienced symptoms), the extent to which these widely prescribed antibiotics increase the risk for melanoma has not been examined. Studies in animals and humans have suggested that other photosensitizers (flouroquinolones, psoralens) enhance UV-associated skin carcinogenesis. The objective of this case control study was to explore the association between tetracycline antibiotics and risk of melanoma in a cohort of White and Hispanic White male patients seen at a Florida veterans' hospital from 1994 to 2000.

After controlling for age, marital status, prisoner of war status, HIV status, combat duty history, branch of service, risk year group and other known photosensitizing drugs using logistic regression analysis, an adjusted odds ratio for those prescribed a tetracycline versus those who were not was 2.064 (95% C.I.=1.228,3.467). Drug specific exposure analyses, comparing subjects who had only Tetracycline or only Doxycycline to those with no history of any type of tetracycline prescription, revealed a four times greater risk for. melanoma for Tetracycline only users (OR=4.049, 95% C.I.= 1.734, 9.452). A positive association (OR=1.947, 95% C.I.=1.000, 3.789) was also seen with Doxycycline only

users. A dose response relationship was observed between the number of treatment regimens of any tetracycline prescribed and log odds of melanoma ($\chi^2 = 3.6707$, $p = 0.055$; Spearman $\rho = 0.64$, $p < .05$).

The high rate of melanoma in this veteran population warrants investigation of any biologically plausible risk factor. Biologic plausibility, along with a two-fold increase of melanoma associated with the prescribing of tetracyclines discovered in this study, justifies further research into tetracyclines as an additional risk factor for melanoma.

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Disclaimer

The views expressed in this dissertation are those of the author, and do not reflect the official policy or position of the United States Air Force, Department of Defense, or the U. S. Government.

Dedication

This dissertation is dedicated to my husband, Norvin, who has lovingly encouraged my every endeavor. Without his inspiration and motivation, this occasion would have never been conceived nor completed.

Acknowledgments

The members of my committee represent the finest people and academicians I've worked with, and are inspirations to students like me. Drs. Mason, Schwartz, Foulis, and Blair contributed to this dissertation and they will forever be a part of me as I moved toward a career in Epidemiology. To them I am forever indebted to their openhanded knowledge, patience, and understanding. I would also like to acknowledge the University of Florida's College of Public Health for a great educational experience and the United States Air Force for providing me the opportunity to pursue this new career.

Office of Graduate Studies
University of South Florida
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CERTIFICATE OF APPROVAL

This is to certify that the dissertation of

NANCY K. FAGAN

in the graduate degree program of
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Member: Skai W. Schwartz, Ph.D.

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CHAPTER ONE: INTRODUCTION

Background

Skin cancer is the most common form of cancer in the United States (National Cancer Institute, 1998). The incidence of all skin cancers has been rising 4% to 5% each year, and there are now more than 1.3 million new cases diagnosed annually. About 95% of these are basal and squamous cell carcinomas (commonly referred to as "non-melanoma skin cancers"), the remaining 5% are malignant melanoma (Fraser & Hartge, 1996). Even though melanoma is the least common skin cancer, it is the most dangerous type of skin cancer, comprising 79% of all skin cancer deaths (American Cancer Society, 1999b). About 25% of all diagnosed melanomas result in death. The number of cases of melanoma has nearly tripled in the last 4 decades, its incidence increasing at a higher rate than the other skin cancers and faster than that of any other cancer (Rigel, 1997).

In the United States, the number of newly diagnosed melanoma cases in 2001 is predicted to be 51,400, with 7800 deaths (Greenlee, Hill-Harmon, Murray, & et al, 2001). The costs in the Medicare program alone spent to treat melanoma in 1990, was estimated at $1.1 billion (Rigel, 1997a). At the current expected annual increase, melanoma incidence should double approximately every 12 years. Factoring in population growth and inflation, the annual cost of melanoma treatment may well exceed $5 billion by the year 2010.

The rapid rise in incidence has not been fully explained, but in 1992, the International Agency for Research on Cancer (IARC, 1992) concluded that there was sufficient evidence in humans for the carcinogenicity of solar radiation and that it causes cutaneous melanoma (Thompson, 1993). Despite much circumstantial evidence linking melanoma to sun exposure, this correlation has not yet been proven (Council on Scientific Affairs, 1989; Elwood & Jopson, 1997). Animal models have shown photo-carcinogenesis, but evidence is from the microscopic interpretation of tissue. Studies have not consistently shown that melanoma is induced by ultraviolet radiation (UV) alone (Council on Scientific Affairs, 1989; Taylor, Stern, Leyden, & al, 1990).

Unlike the more common forms of skin cancer, melanoma is not restricted to areas of the body with greatest accumulated exposure to sunlight (Armstrong & Kricker, 1995; Council on Scientific Affairs, 1989; Elwood & Jopson, 1997), nor is it consistently associated with occupational exposure to sunlight. Also, in contrast to other skin cancers, which seem to be associated with chronic sun exposures, recent studies show that acute, intense exposures to sunlight, especially exposures that causes erythema and inflammation (sunburn), are more strongly linked to the development of melanoma (Elwood & Jopson, 1997; Gilchrest, Eller, Geller, & Yaar, 1999). Individuals who have had melanoma are twice as likely to have experienced at least one episode of severe sunburn and are more than three times as likely to report several episodes of severe sunburn, as those with no history of the disease.

It is not known what molecular events are involved in the complex physiological processes that give rise to erythema in sunburn (Harber & Bickers, 1989). It is believed that a phototoxic exposure to the skin results in a sunburn reaction that

leads to neoplastic changes, eventually resulting in malignant melanomas. Green et al (Green, 1984) pointed out that painful sunburn is the one factor that indicates, regardless of individual variation in pigmentation, that an acute high-dose of UV has been delivered to the level of the melanocyte.

It is well cited that the tetracycline class of antibiotics greatly increase the erythema response (sunburn-like reaction) an individual will experience with exposure to natural or artificial UV light (Stern, 1998). The extent to which this class of drugs increases the cancer risk in humans has not been established.

This study is based on the hypothesis that the use of tetracyclines, because of their potential to cause clinical and subclinical adverse cutaneous reactions that mimic sunburn reactions, is an additional risk factor for the development of melanoma.

Research Questions

If severe sunburn is a risk factor for melanoma, does use of tetracycline antibiotics, which cause sunburn-like reactions as a result of photosensitization, also increase risk for melanoma?

Is there a dose-response relationship between use of tetracyclines and melanoma?

Is there a difference among the types of tetracyclines and subsequent risk for the development of melanomas?

CHAPTER TWO: REVIEW OF LITERATURE

Overview of the Skin and Skin Cancer

The Skin

The skin is considered the largest organ of the body and has many different functions (National Cancer Institute, 1998; Oncology Channel, 2000). It provides cover and protects the internal organs, defends the body against germs and prevents the loss of too much water and other fluids. The skin sends messages to the brain about heat, cold, touch, and pain. The skin is made of three main layers. Beginning from the outside, they are: the epidermis, the dermis, and the subcutaneous layers.

The epidermis is very thin and serves to protect the deeper layers of skin and the organs. The epidermis itself has three layers: an upper, middle, and bottom layer composed of basal cells. These basal cells divide to form keratinocytes, also called squamous cells, which make a substance called keratin that helps protect the body. Melanocytes are also present in the epidermis and produce the pigment called melanin. Melanin gives the tan or brown color to skin that aids in the protection from the harmful effects of the sun. A layer called the basement membrane separates the epidermis from the deeper layers of skin.

The middle layer of the skin is called the dermis. The dermis is much thicker than the epidermis. It contains hair shafts, sweat glands, blood vessels, and nerves. The deepest layer of the skin, the subcutis, keeps in heat and has a shock-absorbing

effect that helps protect the body's organs from injury. Figure 1 shows the layers and cellular components of epidermis.

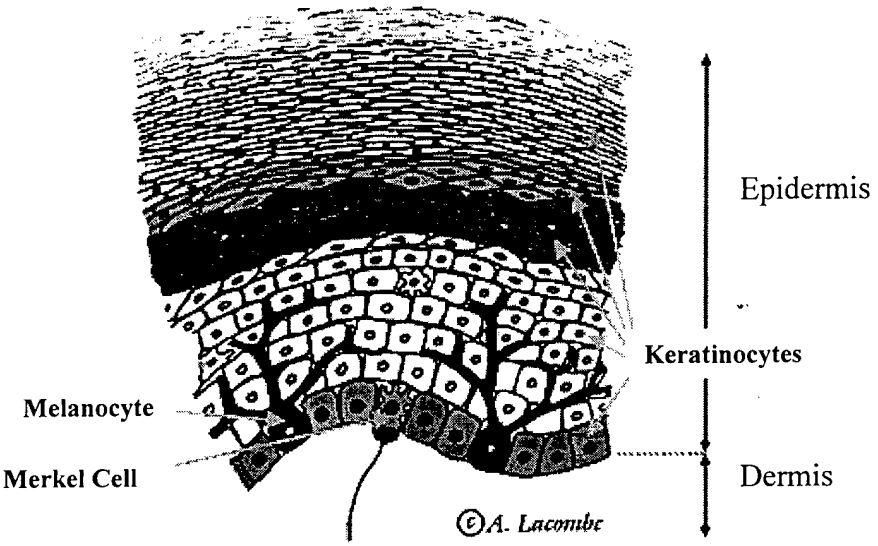


Figure 1. Illustration of the skin's layers and cellular components.

Note: This illustration is from the Department of Zoology, University of British Columbia website, copyright by A. Lacombe, 1997, (Lacombe, 1997).

Differences in human pigmentation are determined by genetic, hormonal, and environmental influences (Lucky & Nordland, 1985). All races are thought to have the same number of melanocytes per unit area of skin, and there do not appear to be differences in the number of keratinocytes with which each melanocyte forms dendritic connections. Although darker races may have a greater proportion of pigment cells that are actively producing melanin, the most important determinants of skin color are the number, size, dispersion, and degree of melanization of melanosomes. The lighter the skin the smaller the melanosomes and vice versa.

In the literature, most studies classify the skin into six specific sun-reactive skin types based on pigmentary characteristics such as skin color and the degree of sensitivity to ultraviolet radiation (UV) (Council on Scientific Affairs, 1989; Gilchrest, 1990). These are: (a) skin type I, always burns easily, never tans; (b) skin type II, always burns easily, tans minimally; (c) skin type III, burns moderately, tans gradually and uniformly (light brown); (d) skin type IV, burns minimally, always tans well (moderate brown); (e) skin type V, rarely burns, tans profusely (dark brown); and skin type VI, never burns, deeply pigmented (black).

For simplicity, skin sensitivity to UV can be divided into three general groups:

(1) Lightly pigmented: UV exposure causes sunburn but little tanning (e.g. Celtic populations). Characteristics of this group include fair or red hair, blue eyes and freckles. People in this group must take extra care in the sun as their skin is poorly protected and easily damaged.

(2) Intermediately pigmented: UV exposure results in little sunburn but tanning always occurs (e.g. southern Mediterranean and Asian populations). Characteristics of this group include darker hair and eyes. Although able to tan, people in this group can still burn and sustain significant skin damage from UV.

(3) Heavily pigmented: UV exposure rarely causes sunburn (e.g. Aboriginal, African and American Negroid populations). These populations have very good natural protection and are at little risk of skin cancer, but are, like all groups, subject to UV-induced eye damage and possibly reduced ability to combat infections when exposed to excessive UV levels.

Skin cancer

Cancer of the skin is the most common of all cancers (National Cancer Institute, 1998). Like other forms of cancer, skin cancers are normal cells which in some way are altered (i.e. genetically or biochemically) in a way that permits them to divide into many more cells than would normally be present. Abnormal growth leads to the development of skin cancers in the outermost protective layer of the skin, the epidermis. Each component of the epidermis can give rise to benign and malignant neoplasms: keratinocytes can give rise to squamous cell and/or basal cell carcinomas and melanocytes can give rise to nevi (benign neoplasms) and malignant melanomas. Skin cancers are divided into two major groups based on their cellular origin: melanoma skin cancer and nonmelanoma skin cancer.

Nonmelanoma skin cancers (basal cell and squamous cell cancers) are the most common cancers of the skin. They are called nonmelanoma because they develop from skin cells other than melanocytes. Squamous cell carcinomas (SCCs) come from altered, squamous cells in the outer layers of the epidermis. Basal cell carcinomas (BCCs) come from changed cells of the innermost or basal layer of the epidermis.

Melanoma is a cancer that begins in epidermal cells called melanocytes. Melanocytes are epidermal cells that produce the skin coloring. Other names for melanoma include malignant melanoma and cutaneous melanoma. Melanoma is much less common than basal cell and squamous cell skin cancers, and it is almost always curable in its early stages, but it is much more likely than basal or squamous cell cancer to metastasize to other parts of the body and cause death. Because most cancerous

melanoma cells continue to produce melanin, melanoma tumors are often brown or black.

The Disease of Interest: Melanoma

Epidemiology

Malignant melanoma continues to present a significant public health problem as its incidence is rising faster than that of any other cancer in the United States (American Cancer Society, 1999a). A review of melanoma incidence and mortality data since the 1950s shows a consistent 6% annual increase in incidence and a 2% annual increase in mortality (Rigel, 1997a).

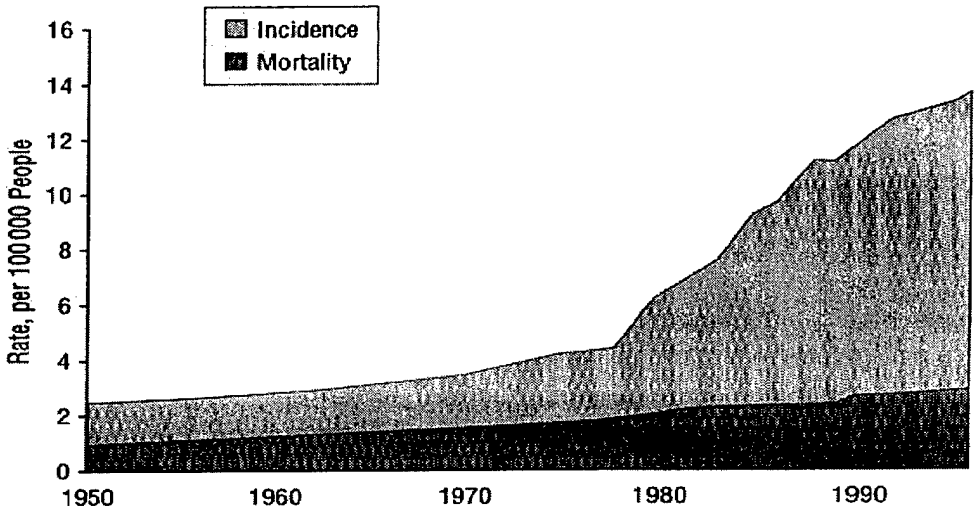


Figure 2. Incidence and mortality rates (per 100,000 people) for melanoma in the U.S., 1950-1996.

Note: From "Melanoma incidence: if it quacks like a duck," by D. Rigel, 1997, *Archives of Dermatology, 133*(5), p. 656-658.

According to the American Cancer Society, at these current rates, 1 in 75 Americans born in 2000 will develop melanoma during his or her lifetime. Figure 3 represents the increase in lifetime risk of Americans since 1935 (Rigel, 1997b).

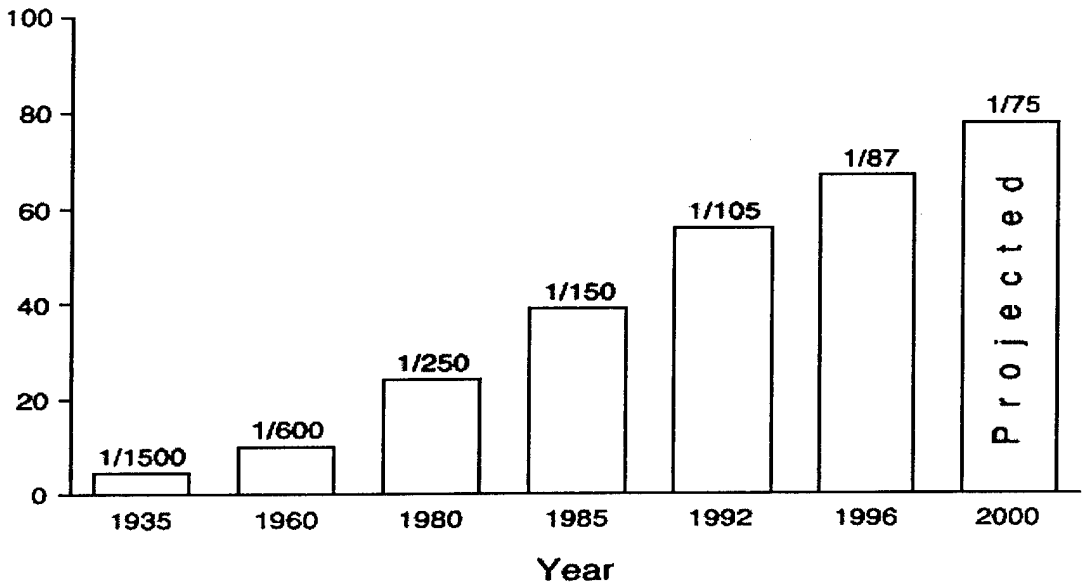
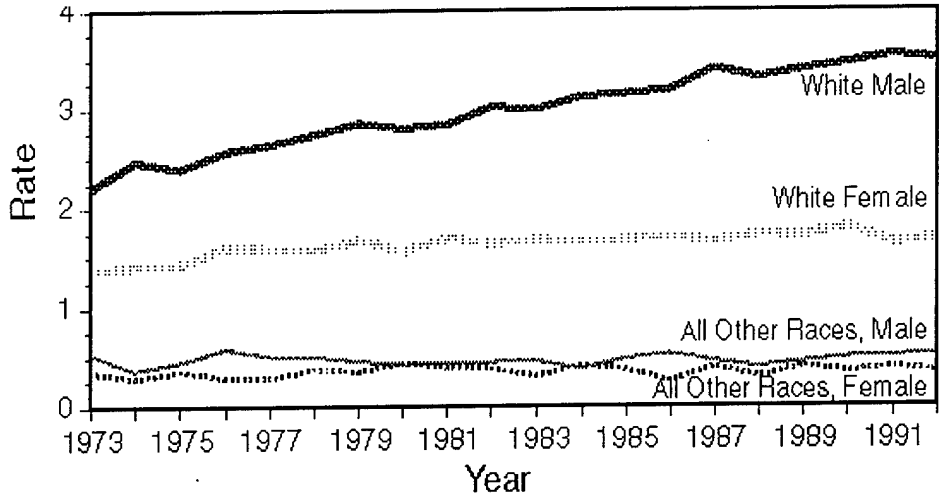


Figure 3. Lifetime risk for development of melanoma in citizens of the United States. Note. From "Malignant melanoma: incidence issues and their effect on diagnosis and treatment in the 1990s", by D. Rigel, 1997, *Mayo Clinic Proceedings*, 72(4), p. 367-371.

Although accounting for only 5% of skin cancer cases, melanoma skin cancers are the cause of 79% of all skin cancer deaths (American Cancer Society, 1999b; Fraser & Hartge, 1996). According to the Melanoma Research Foundation (Melanoma Research Foundation, 2001), every hour of every day of the year, a human being dies of melanoma in the United States. Melanoma strikes people of all ages, all races, all economic levels and both sexes. It is most common in people of Caucasian descent (Fraser & Hartge, 1996). Figure 4 shows the annual age adjusted rate of deaths from

melanoma by race and sex based on data collected from the Surveillance, Epidemiology, and End Results (SEER) Program from 1973 to 1992.



*Per 100,000 population, adjusted to the 1970 U.S. population.

Figure 4. Average annual age adjusted rate* of deaths from melanoma, by race and sex – United States 1973-1992.

Note. From the SEER Program, by Ries LAG, Kosary CL, Hankey BF, Miller BA, Clegg L, Edwards BK (eds). SEER Cancer Statistics Review, 1973-1996, National Cancer Institute. Bethesda, MD, 1999.

The increased rates among whites have been shown to increase with age in both men and women. The size of the increase is over three fold in men and about 62% in women (Dennis, 1999). Figure 5 shows the age-adjusted rates of melanomas for white men and women found in the SEER database as calculated by L.K. Dennis (1999b).

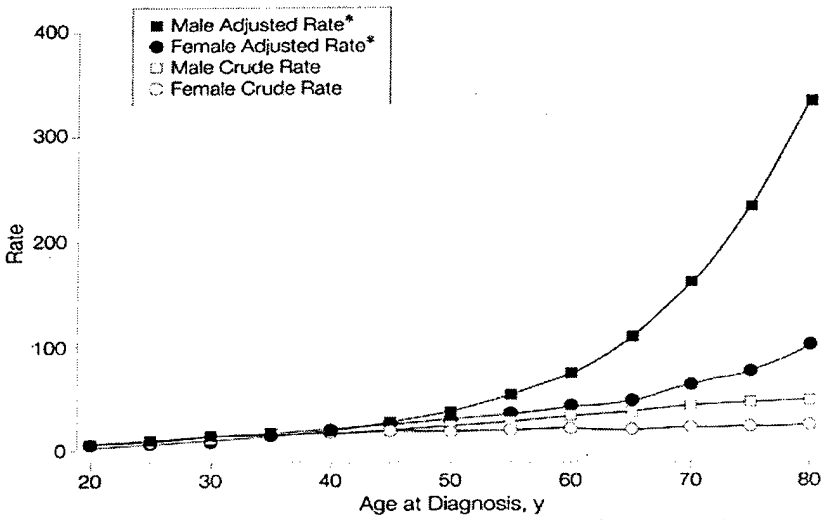


Figure 5. Surveillance, Epidemiology, and End Results (SEER) Program Melanoma Incidence Crude and adjusted age-specific incidence rates* (per 100,000 population) of all melanomas for men and women, 1973-1994 SEER data adjusted for birth-cohort effects. *adjusted for birth cohort.

Note. From "Increasing risk of melanoma with increasing age", by L.K. Dennis, 1999, *JAMA*, 282(11), p.1037-1038.

Jemal et al (Jemal, Devesa, Hartge, & Tucker, 2001) also found that between 1973 and 1997, the incidence of melanoma almost tripled among males, and more than doubled among females.

According to Klein et al (Klein & Stockford, 2000), one in four U.S. adult males are veterans and over 88.9% of veterans are White. One might expect a higher rate just based on the majority of male gender and White ethnicity in this population. Using age-adjusted rates from SEER data, expected rates were calculated for the James A. Haley Veterans' Hospital male patient population to compare this veteran population to the male SEER population. Figure 6 shows the calculated expected and observed age specific rates using the SEER age-adjusted rates as the standard rate.

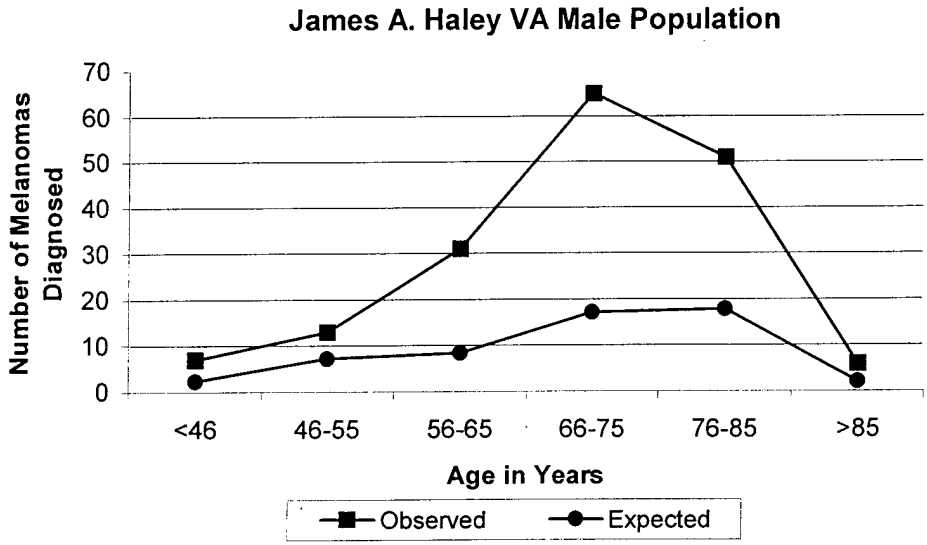


Figure 6. Comparison of expected and observed rates in the James A. Haley Veterans' Hospital population using male SEER data as a standard population (1994-1998).

As seen in Figure 6, the male veteran population at James A. Haley Veterans Hospital had up to three times more cases of melanomas diagnosed than what would be expected based on those men followed in the SEER population during the 1994 through 1998. This extremely high numbers in the 55-85 year olds has yet to be completely explained by any previous published studies.

Impact on Healthcare Resources

In the United States, the number of newly diagnosed melanoma cases in 2001 is predicted to be 51,400, with 7800 deaths (Greenlee et al., 2001). The implications of this increasing incidence in terms of costs to the both the VA and national healthcare systems are overwhelming. In 1990, an estimated $1.1 billion was spent on melanoma treatments in the Medicare program alone (Rigel, 1997a). At the current expected annual increase, melanoma incidence should double approximately every 12 years with

the annual cost of melanoma treatment well exceeding $5 billion by the year 2010 (factoring in population growth and inflation).

Origin

Melanoma is the result of neoplastic transformation of melanocytes (WHO, 1999), the cells in the epidermis that produce the pigment melanin that colors our skin, hair, and eyes and is heavily concentrated in most moles (Lucky & Nordland, 1985). The melanocytes arise from cells migrating from the neural crest and are found in the basal layer of the epidermis, and in hair follicles. The cells are dendritic in appearance with vacuolated cytoplasm. Depending on anatomic location, approximately one in every 10 basally located cells is a melanocyte, each of which produces melanin for approximately 36 keratinocytes. Melanocytic nevi are pigmented, benign neoplasms composed of modified melanocytes (nevus cells); they may be congenital or acquired lesions. In the average person with no nevi present at birth, there will be approximately 24 nevi acquired by the age of 24 years.

An amino acid, tyrosine, is converted to melanin through a series of complex chemical steps in the skin cells (Harber & Bickers, 1989). This process may be affected by heredity, heat, trauma, solar or ionizing radiation, heavy metals, and other factors. Pigment production and distribution in the body is controlled by melanocyte-stimulating hormone (MSH) from the pituitary gland. Changes in any of these factors can result in hyperpigmentation (increase in pigment production), hypopigmentation (decrease in pigment production), or both. The changes may be temporary or permanent. Pigment changes can be primary (existing as a separate disorder) or

secondary to other disorders. A person's degree of skin pigmentation determines, to some extent, the various dermatological diseases to which a person may be susceptible.

Melanosomes, pigment-containing granules produced within melanocytes, are present in melanocyte dendrites and are transferred to surrounding keratinocytes. The melanocytes tend to form a cap over the keratinocyte nucleus and partially protect it from UV radiation. Some pigment cells fail to remain in their basal destination, instead re-entering the dermis as nests of pigment cells. These nests form the melanocytic nevi or moles that may be acquired during postnatal life or may be congenital. Some uncommon melanocytic nevi arise by failure of migration into the epidermis.

Pathology

One way to categorize malignant skin melanomas is by their growth pattern or morphology (Harber & Bickers, 1989; Oncology Channel, 2000). The four types of growth patterns are superficial spreading melanoma (SSM), nodular melanoma (NM), lentigo maligna melanoma (LMM) and acral lentiginous melanoma (ALM).

Superficial spreading melanoma (SSM) accounts for about 70% of all melanomas. This type of melanoma usually originates in a pre-existing nevus. It is a slow-growing (1-5 years) melanoma that can occur any time after puberty.

Nodular melanoma (NM) accounts for about 15-30% of all melanomas. NMs are more aggressive and grow more rapidly than SSM's. It usually develops in middle age and is most common on the trunk or head and neck area. NMs usually develop on normal skin and not from a pre-existing nevus. NMs are small (1-2 cm) and typically blue-black in color and resemble blood blisters.

Lentigo maligna melanoma (LMM) accounts for only 4-10% of all melanomas. They differ from other melanomas in that they tend not to metastasize. They are typically located on the face in older individuals and are most often found in Caucasian women. When diagnosed, they have usually been present for 5-15 years. They are large (greater than 3 cm) and flat and usually tan in color. As they enlarge, they may have a mottled appearance. They have irregular borders with lots of notching and indentation.

Acral lentiginous melanoma (ALM) composes 7-9% of all melanomas but may account for up to 60% of melanomas in dark-skinned patients. This pattern typically occurs on the hands, feet, beneath the nail beds, mucous membranes. They are usually large (greater than 3 cm) and typically resemble LMMs in their early stages.

Staging

Microstaging is an integral part of the staging and clinical management of melanoma. Two methods have been used (Rigel, Kopf, & Friedman, 1987). The Breslow microstaging method measures the thickness of the lesion in millimeters using an ocular micrometer. The total vertical height of the melanoma is measured from the granular layer to the area of deepest penetration. If the lesion is ulcerated, measurements are made from the surface of the ulcer to the deepest part of the lesion. The Clark method assesses the level of penetration into the various skin layers: (a) Level I, confined to the epidermis (in situ); (b) Level II, invasion into the papillary dermis; (c) Level III, penetration to the papillary-reticular interface; (d) Level IV, invasion into the reticular dermis; and (e) Level V, penetration into subcutaneous fat.

Although the tumor thickness and the level of invasion can predict the risk for metastases, tumor thickness may be a more accurate and reproducible prognostic parameter than interpreting the level of invasion. Significant regression of the tumor invalidates the prognostic value of these microstaging methods.

A four-stage system developed by the American Joint Committee for Cancer (AJCC) is currently used to stage melanoma. It takes into consideration the thickness of the tumor, how deeply it has invaded the skin, and whether the disease has spread to nearby lymph nodes or distant organs. Clinical staging criteria can be found in Table 1.

Table 1.

AJCC Cancer Stage Criteria as Compared to Clark Level Staging Criteria.

Stage	Description	Depth	Clark Level
0	Melanoma in situ	Only in top layer of epidermis	I
IA	Localized melanoma	<0.75 mm	II
IB	Localized melanoma	0.76 to 1.5 mm	III
IIA	Localized melanoma	1.5 to 4 mm	IV
IIB	Localized melanoma	>4 mm	V
III	Limited nodal metastases involving only one regional lymph node basin, or fewer than 5 in-transit metastases without nodal metastases		
IV	Advanced regional metastases or any patient with distant metastases		

The stage of tumor determines the treatment required (American Cancer Society, 1999b; National Cancer Institute, 1999b). The standard treatments are:

1. Stage 0 - Melanoma in situ, found only on the top layer of the epidermis is virtually always curable when treated by surgery.

2. Stage IA - This tumor is considered low-risk, less than .75 mm 1/16 inch in thickness, and/or has not penetrated to the papillary dermis, the skin layer immediately

under the epidermis. The tumor has not spread to the lymph nodes or distant organs. This tumor is treated surgically.

3. Stage IB - This stage tumor is low-risk, between .75 mm and 1.5 mm 1/6 inch thick and/or has penetrated to the reticular dermis, the layer of skin under the epidermis. Treatment is by surgery.

4. Stage IIA - This melanoma is between 1.5 mm and 4 mm and/or has penetrated the lower reticular dermis, or deep dermis. Treatment is by surgery.

5. Stage IIB - This tumor is thicker than 4 mm and/or invades the subcutaneous fat. Additional tumors called "satellites" may be found within 2 centimeters of the original tumor. Treatment is by surgery.

6. Stage III - This melanoma has spread to the lymph node associated with the affected skin area. Treatment is by surgery. In most cases, adjacent lymph nodes are also removed, and biological therapy with interferon may be recommended.

7. Stage IV - The melanoma has spread to other organs such as the lung, liver, or brain, or to distant areas of skin or lymph nodes. Treatment may include surgery, chemotherapy, and biological therapy with interleukin-2.

A revised TNM staging was proposed at the Fifth World Conference on Melanoma in February 28-March 3, 2001 and is illustrated in Appendix A (Mocharnuk, 2001). It is expected to be adopted formally in 2002.

Prognosis

According to Rigel et al (Rigel et al., 1987), many factors are known to predict risk in patients with melanoma including Breslow depth, Clark level, anatomic location, gender, tumor ulceration, and growth pattern. The higher the stages of melanoma, the

lower are the chances for cure or long-term survival. Stage I and II patients demonstrate that tumor thickness is the single most important prognostic factor. Other important risk factors include the presence of ulceration and anatomic location, with extremity lesions having a better prognosis than those on the trunk or head and neck. Stage III patients show that the number of metastatic nodes, the anatomic site of the primary lesion, and tumor ulceration are the dominant prognostic variables. According to the American Cancer Society (1999b), the 10-year survival rate for stage I melanoma is 85% to 90%. The 10-year survival rate for stage II is about 60%, and about 25% for stage III. Only about 10% of people with stage IV melanoma survive 3 years after diagnosis (American Cancer Society, 1999b).

Clinical Signs of Melanoma

Often, the first sign of melanoma is a change in the size, shape, color, or feel of an existing mole (Silberman, 2001). Most melanomas have a black or blue-black area. Melanoma also may appear as a new, black, or abnormal mole. Benign skin lesions can usually be differentiated from malignant ones. The American Cancer Society, the American Academy of Dermatology, and the Skin Cancer Foundation have devised a simple ABCD rule to help people remember some of the signs of melanoma. This mnemonic is illustrated in Figure 7.

Factor	Description
Asymmetry	One half of the mole or growth does not match the other half.
Border irregularity	The edges are ragged, notched or blurred.
Color	The pigmentation is not uniform. Shades of tan, brown, and black may be present. Red, white, and blue may add to the mottled appearance.
Diameter	Any mole larger than 6 mm (the size of a pencil eraser) or exhibiting a sudden or continuing increase in size should be of special concern.

Figure 7. A, B, C, and D's for recognition of early malignant melanoma.

Note. Adapted from "Malignant melanoma: incidence issues and their effect on diagnosis and treatment in the 1990s", by D. Rigel, 1997, *Mayo Clinic Proceedings*, 72(4), p.367-371.

Melanomas can vary greatly in the ways they look. Many show all of the ABCD features. However, some may show changes or abnormalities in only one or two of the ABCD features. Early melanomas may be found when a pre-existing mole changes slightly--such as forming a new black area. Other frequent findings are newly formed fine scales or itching in a mole. In more advanced melanoma, the texture of the mole may change. For example, it may become hard or lumpy. Although melanomas may feel different and more advanced tumors may itch, ooze, or bleed, melanomas usually do not cause pain.

Melanoma most often appears on the trunk of fair-skinned men and on the lower legs of fair-skinned women, but it can appear other places as well. Having darkly pigmented skin lowers the risk of melanoma but it does not mean that a person with dark skin will not develop melanoma. People with darker skin can have this cancer on the palms of the hands, soles of the feet, and under the nails. Rarely, melanomas can form in parts of the body not covered by skin such as the eyes, mouth, vagina, large intestine, and other internal organs.

Risk Factors

What causes melanoma? The answer to that question is still under investigation. Numerous clinical and epidemiologic studies have identified characteristics associated with increased risk of melanoma. These risk factors include personal, family-related, and environmental aspects, and/or a combination of the three (American Academy of Dermatology, 2000). Some risk factors studied include, but are not limited to: (a) UV radiation - to include sun, artificial light, sunburn; (b) Nevus phenotype (dysplastic, type and density); (c) Phenotypic characteristics/sun sensitivity; (d) Family history of melanoma; (e) Personal history of previous melanoma; (f) Congenital melanocytic nevi; (g) Immunosuppression; and (h) Pharmaceuticals.

Ultraviolet Radiation (UV)

The process by which UV radiation is associated with the development of the disease is not well understood (National Cancer Institute, 1998; Fraser & Hartge, 1996). Many researchers believe the damage caused by UV radiation causes changes in the deoxyribonucleic acid (DNA) within melanocyte skin cells, eventually giving rise to melanoma. This is either by chronic or acute (sunburn) exposure.

Solar radiation and artificial light sources are the primary sources of UV radiation that elicit biological effects in the skin. Ultraviolet refers to all electromagnetic radiation with wavelengths in the range of 10 to 400 nanometers, or frequencies from 7.5E14 to 3E16 Hz (Harber & Bickers, 1989). Scientists classify UV radiation into three types or bands - UVA, UVB, and UVC. The stratospheric ozone layer absorbs some, but not all, of these types of UV radiation limiting the amount to which the body is exposed.

UVA rays constitute 90-95% of the ultraviolet light reaching the earth (Committee on Environmental Health & American Academy of Pediatrics, 1999). They have a relatively long wavelength (320-400 nm) and are not absorbed by the ozone layer. Wavelengths from about 345 to 400 nM are used for "Blacklight" effects and are very slightly visible if isolated from more visible wavelengths. Shorter UVA wavelengths from 315 to 345 nM are used for suntanning. UVA rays penetrate deeper into the skin and are strongly absorbed by the melanocytes, which are involved both in melanin production (sun tanning) and in melanoma formation (Harber & Bickers, 1989). Most chemical sunscreens were only formulated to absorb UVB radiation.

UVB rays are partially absorbed by the ozone layer and have a medium wavelength (290-320 nm). They do not penetrate the skin as far as the UVA rays do and are the primary cause of sunburn. They are also responsible for most of the tissue damage which results in wrinkles and aging of the skin and are implicated in cataract formation.

UVC rays have the shortest wavelength (200 to 290 nm) and are almost totally absorbed by the ozone layer. Wavelengths in the UVC range, especially from the low

200's to about 275 nM, are especially damaging to exposed cells. As the ozone layer thins UVC rays may begin to contribute to sunburning and premature aging of the skin.

All forms of ultraviolet radiation are believed to contribute to the development of skin cancer. Figure 8 shows the depth to which UV radiation penetrates the layers of the skin.

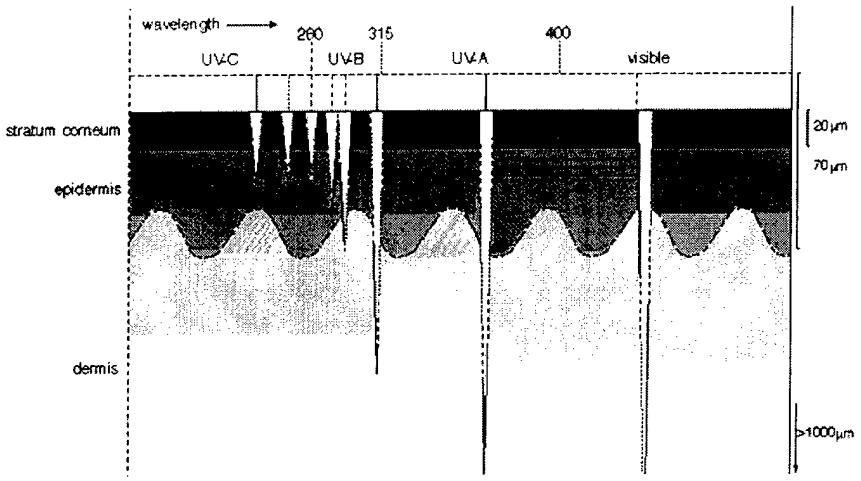


Figure 8. A cross-section of the human skin and underlying tissue, showing the depth to which UV radiation penetrates.

Note. From "Impacts of a Projected Depletion of the Ozone Layer", by F.R. deGruijl, *Consequences*, 1995, 1 (2).

The level of UV radiation that reaches the Earth's surface can vary, depending on a variety of factors: (EPA, 1999)

1. Stratospheric Ozone: The ozone layer absorbs most of the sun's UV rays, but the amount of absorption varies depending on the time of year and other natural phenomena. That absorption also has decreased, as the ozone layer has thinned due to the release of ozone-depleting substances that have been widely used in industry.

2. Time of Day: The sun is at it's highest in the sky around noon. At this time, the sun's rays have the least distance to travel through the atmosphere and UVB levels are at their highest. In the early morning and late afternoon, the sun's rays pass through the atmosphere at an angle and their intensity is greatly reduced.

3. Time of Year: The sun's angle varies with the seasons, causing the intensity of UV rays to change. UV intensity tends to be highest during the summer months.

4. Latitude: The sun's rays are strongest at the equator, where the sun is most directly overhead and UV rays must travel the least distance through the atmosphere. Ozone is naturally thinner in the tropics compared to the mid- and high-latitudes, so there is less ozone to absorb the UV radiation as it passes through the atmosphere. At higher latitudes the sun is lower in the sky, so UV rays must travel a greater distance through ozone-rich portions of the atmosphere and, in turn, expose those latitudes to less UV radiation. There is an inverse relationship between latitude and the incidence and mortality rates of melanoma in whites, with higher rates seen closer to the equator (where there is a greater amount of sunlight) (Kopf, Kripke, & Stern, 1984).

5. Altitude: UV intensity increases with altitude because there is less atmosphere to absorb the damaging rays. Thus, when you go to higher altitudes, your risk of overexposure increases.

6. Weather Conditions: Cloud cover reduces UV levels, but not completely. Depending on the thickness of the cloud cover, it is possible to burn - and increase your risk of long-term skin and eye damage - on a cloudy summer day, even if it does not feel very warm.

7. Reflection: Some surfaces, such as snow, sand, grass, or water can reflect much of the UV radiation that reaches them. Because of this reflection, UV intensity can be deceptively high even in shaded areas.

The above factors, plus alterations in lifestyles of large portion of populations, including travel, clothing style changes, and the beauty of tanning in current day societies make it a challenge to study the harmful effects of the UV radiation.

Sun exposure. Most of what is known about the links between sunlight and melanoma comes from case-control studies in which individuals are asked to recall previous sun exposure (Diffey & Gies, 1998). The evidence described in the literature has been weak and/or conflicting. Even though the literature is not convincing, reputable groups such as the International Association have accepted sun exposure as a causal factor of cutaneous melanoma in humans (IARC, 1992).

The complication with measuring sun exposure, unlike an exposure such as smoking, is that the same question and response in different studies will relate to a different dose of solar UV radiation because of the confounding of behavior outdoors with ambient UV levels (Diffey & Gies, 1998).

Descriptive epidemiological studies and clinical observations in the early 1980s led to the hypothesis that melanoma is related specifically to intermittent sun exposure, in contrast to other types of skin cancer, which were assumed to be related to continued or chronic exposure (Elwood & Hislop, 1982; Holman, Armstrong, & Heenan, 1983).

In 1997, Elwood and Jobson (1997) attempted to summarize the results of the published case control studies in an effort to elucidate a consensus of findings on the relationship between sun and melanoma. Through the systematic review of thirty five

published case-control studies results (several with more than one publication), they tried to assess the association between the incidence of cutaneous melanoma and intermittent, occupational and total sun exposure; and history of sunburn at different ages (Autier et al., 1994; Chen et al., 1996; Cristofolini et al., 1987; Dubin, Pasternack, & Moseson, 1990; Dunn-Lane, Herity, Moriarty, & Conroy, 1993; Elwood, Whitehead, Davison, Stewart, & Galt, 1990; Green, 1984; Grob et al., 1990; Holly, Aston, Cress, Ahn, & Kristiansen, 1995; Holly, Kelly, Shpall, & Chiu, 1987; Holman, Armstrong, & Heenan, 1986; Lew, Sober, Cook, Marvell, & Fitzpatrick, 1983; MacKie & Aitchison, 1982; MacKie, Aitchison, & Freudenberger, 1989; MacKie, Freudenberger, & Aitchison, 1989; Osterlind & Jensen, 1987; Rodenas, Delgado-Rodriguez, Herranz, Tercedor, & Serrano, 1996; Weinstock et al., 1989; Westerdahl, Olsson, & Ingvar, 1994; Zanetti, Franceschi, Rosso, Colonna, & Bidoli, 1992).

The analysis was based on a total of 9,121 incident melanoma cases. Twenty-nine studies contributed data on sun exposure. Overall, there was a significant positive association (odds ratio (OR) = 1.71) for intermittent exposure, a significantly reduced risk for heavy occupational exposure (OR = 0.86) and a small, marginally significant excess risk for total exposure (OR = 1.18).

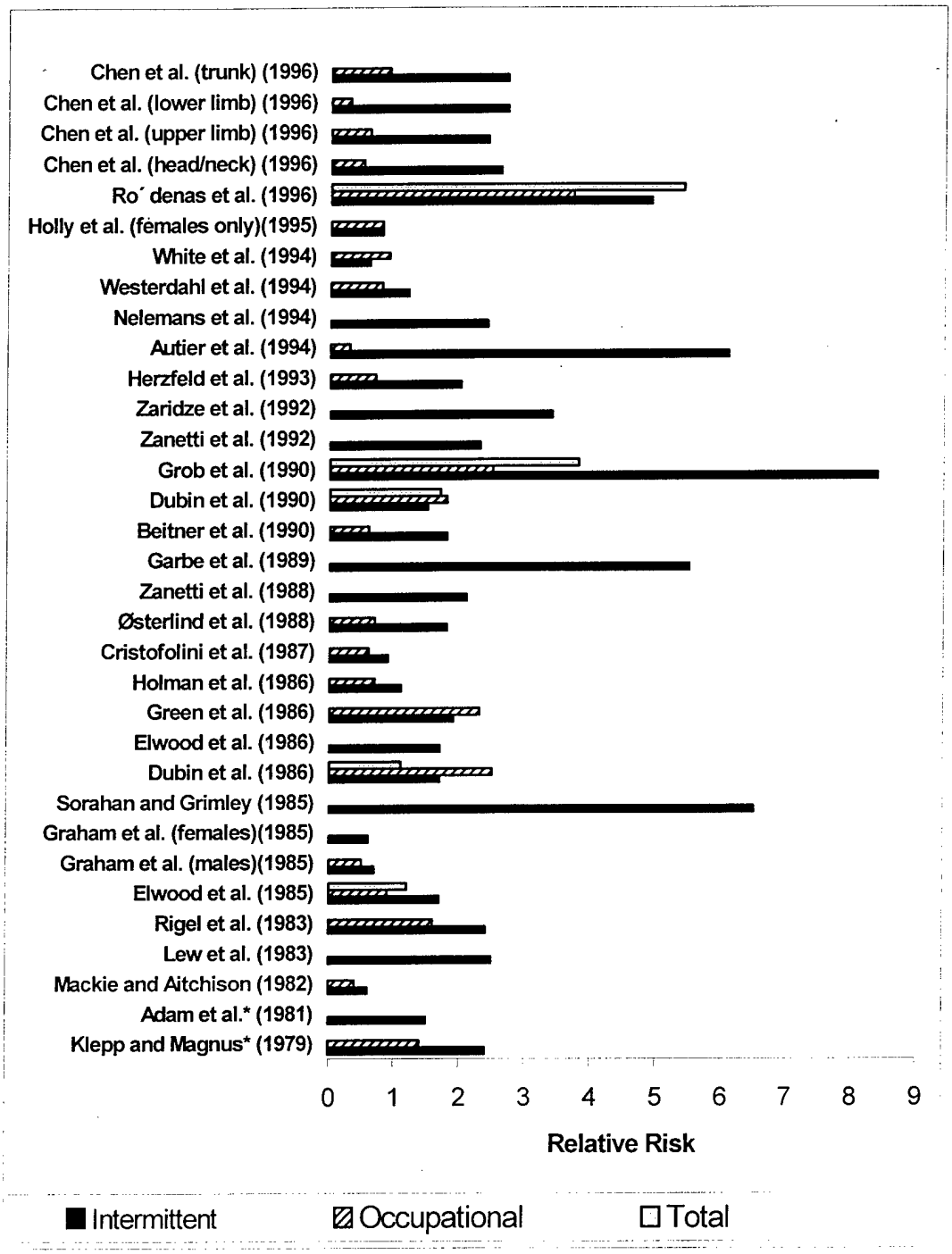


Figure 9. Results of case-control studies on sun exposure and cutaneous melanoma using findings from Elwood and Johnson et al (1997).

Note. * =result was not significant.

Artificial sources of radiation (Longstreth, 1987). The most common sources of artificial UV radiation exposure are various kinds of lamps that emit this form of energy (National Institute of Health, 1989). These lamps are used primarily for recreational tanning and phototherapy of skin diseases (e.g., psoriasis and cutaneous T-cell lymphoma, mycosis fungoides). UVR lamps can emit UVA, UVB, and/or UVC. Those lamps currently used for recreational tanning emit UVA primarily or exclusively. The use of artificial ultraviolet sources for the phototherapy of dermatologic diseases has increased substantially in recent years and has exposed a group of people to markedly increased doses of UVR. Some patients on this type of therapy have shown an increased risk of nonmelanoma and melanoma skin cancer (Gupta & Anderson, 1987). Exposure to sun beds and sunlamps, which produce primarily UV-A, has also been associated with increased risk of melanoma in some studies (Westerdahl, Olsson, Masback et al., 1994). In Sweden, people less than 30 years of age who used sun beds or sunlamps greater than 10 times a year had an almost eightfold increased risk for developing melanoma (Westerdahl, Olsson, Masback et al., 1994).

Another potential source of artificial UVR is unshielded fluorescent bulbs used for illumination. No studies were found that showed association of these bulbs with melanoma risk (Longstreth, 1987).

Sunburn. Green et al (1984) consider chronic and acute exposures to sun to be two distinct entities and present different risks for melanoma. They believe that an experience of painful erythema more accurately indicates that an acute high-dose UV has been delivered to the level of the melanocyte that is capable of causing damage to the cell's DNA.

Sunburn results when the amount of exposure to the sun or other ultraviolet light source exceeds the ability of the body's protective pigment, melanin, to protect the skin (Harber & Bickers, 1989). First and second-degree burns can be sustained from sun exposure. First-degree burns affect the outer layer of the skin, causing pain, erythema (abnormal redness of the skin due to dilation of the superficial capillaries of the skin causing inflammation), and swelling. Second-degree burns affect both the outer and underlying layer of the skin, causing pain, erythema, swelling, and blistering. Sunburn in a very light-skinned person may occur in less than 15 minutes of noonday sun exposure, while a dark-skinned person may tolerate the same exposure for hours.

Sunburn is a cutaneous photosensitivity reaction that can be elicited in virtually all humans and is the most frequently occurring phototoxic reaction, requiring only the interaction of ultraviolet light with skin. Sunburn erythema can be evoked by radiation from all three regions of the UV spectrum (Larsen, 1994). The doses required to elicit minimal erythema in human skin differ considerably among the three types of radiation. In the past it was generally believed that in addition to being the most potent segment of the solar ultraviolet spectrum in causing erythema and tanning, UVB was primarily responsible for the damaging effects of prolonged sun exposure, such as premature aging of skin actinic keratosis and skin carcinogenesis. However, studies have shown that UVA may exert similar effects in mammalian skin (Staberg, Wulf, Poulsen, Klemp, & Brodthagen, 1983). Although UVA is 1000fold less potent than UVB in producing erythema in human skin, its predominance in the solar spectrum reaching the earth's surface (10- to 100-fold more than UVB) may permit it to play a far more important role in the toxic effects of sunlight than hereto fore suspected.

Thus, it is conceivable that as much as 10% of the biologic consequences of sun exposure may be due to UVA. The relative contribution of each is still unknown (Harber & Bickers, 1989).

In the Elwood and Jopson (1997) analysis, there were 21 studies that contributed information on sunburn. There was a significantly increased risk with sunburn at all ages or in adult life (OR = 1.91) and similarly elevated relative risks for sunburn in adolescence (OR = 1.73) and in childhood (OR = 1.95).

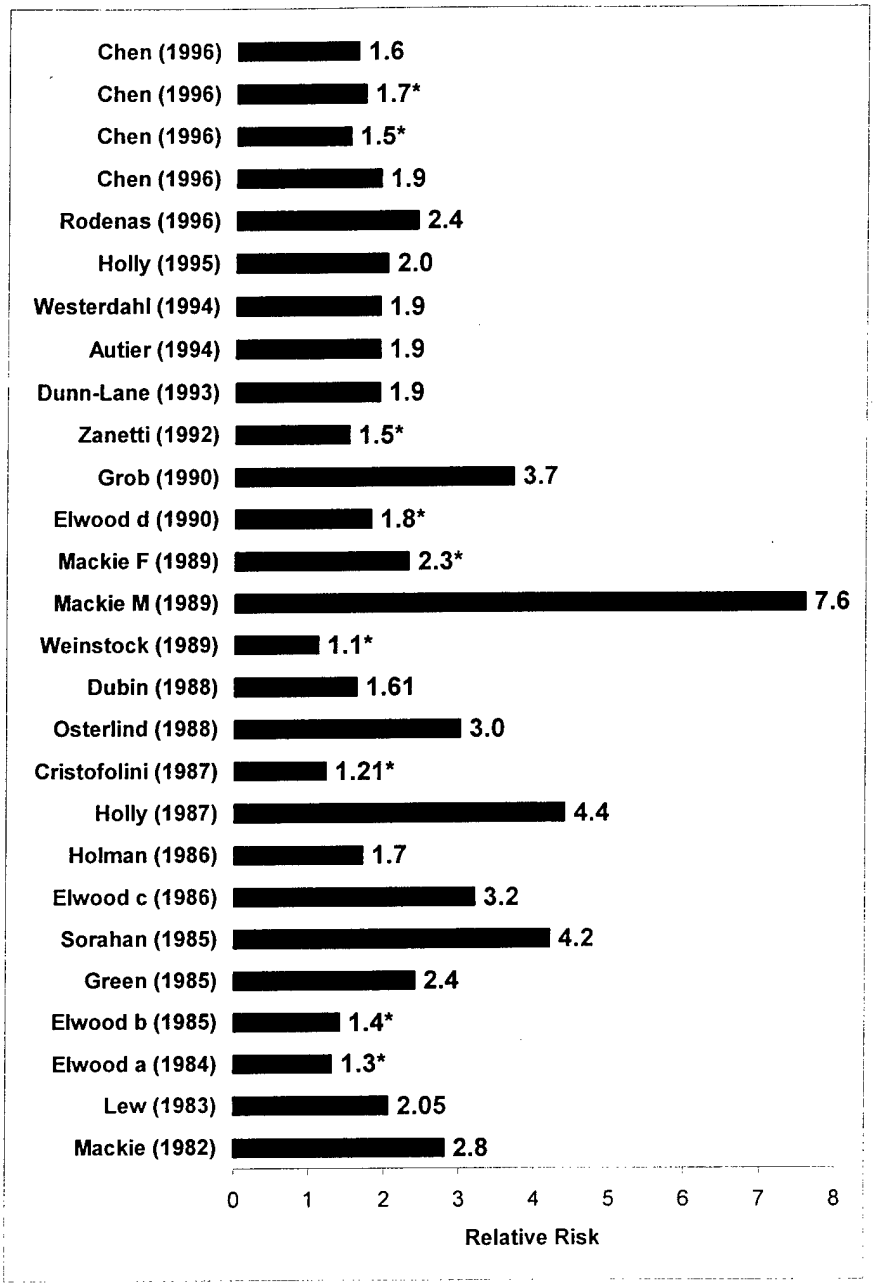


Figure 10. Summary of sunburn studies reviewed by Elwood and Jopson (1997).

Note: * means the results were not significant at the .05 level.

In Elwood and Jopson's review they reported a clear and significant positive association between melanoma and a history of sunburn. Sunburn is usually caused by unaccustomed severe sun exposure and maybe just another indicator of an intermittent

exposure pattern. The strength of the association was similar to that of intermittent exposure. The odds ratios (ORs) for sunburn in childhood (OR=1.95), in adolescence (OR=1.73) and in adulthood or lifelong (OR=1.91) are similar, though a comparison of ORs for different exposure variables needs to allow for differences in reliability of the questions used. This and another study (Whiteman & Green, 1994) found that there was no major difference in effects by age.

The mechanisms by which sunburn increases risk, while other patterns of exposure do not, is described by Gilchrest et al (1999). This group, like Green et al, believes a typical sunburn damages the DNA of the melanocytes located deeper in the skin. They proposed a theory for why the risk of melanoma rises directly with the number of sunburns incurred and not with chronic sun exposure like nonmelanoma skin cancers.

The authors' speculate that nonmelanoma skin cancers are less likely to occur with sunburns because the outer cells of the skin are more vulnerable to the sun and they quickly undergo a process called apoptosis, or programmed cell death, which eliminates cells with damaged DNA. The cells are eventually shed from the skin. This is the peeling process after a sunburn.

The melanocytes, on the other hand, respond to acute sun exposure quite differently. The melanocytes' reaction to injury posed by UV radiation is to transfer more melanin to the surrounding outer skin cells to protect the skin. Research shows melanocytes are highly resistant to programmed cell death. This may be nature's way of assuring the continued presence of protective melanin pigment in the skin. This

resistance to cell death enables UV-damaged melanocytes to remain to mutate and divide, increasing the risk of melanoma with each sunburn.

Phenotypic Characteristics/Sun Sensitivity

For superficial spreading melanomas (SSMs) and nodular melanomas (NMs) (two of four different types of melanoma), susceptibility to sunburn may be just as important as whether or not a person has ever actually had a sunburn (Oncology Channel, 2000). So, people with light skin and who freckle easily, for example, whether or not they have ever actually experience a sunburn, are at an increased risk for developing SSMs and NMs. For these people, it is not clear what actually causes the melanoma. Also, many melanomas develop in areas that are not exposed to the sun, such as the soles of the foot. This is especially true for non-white people.

Numerous epidemiologic studies have focused on identification of important pigmentary characteristics and propensity to burn rather than tan (or sun sensitivity), in the etiology of melanoma (Elwood et al., 1984; Green & O'Rourke, 1985; Holman & Armstrong, 1984; Naldi, Lorenzo Imberti, Parazzini, Gallus, & La Vecchia, 2000; Osterlind et al., 1988). In Longstreth's (Longstreth, 1987) review of studies dealing with phenotypes, she listed the following constitutional characteristics as risk factors for melanoma:

1. Skin color - epidemiologic evidence from several countries is consistent and shows a clear-cut difference between white and non-white races in the incidence of melanoma. Fair complexions relative to dark complexions were associated with elevated risks of melanoma in all studies reviewed. Caucasian melanoma incidence rates in the U.S. are approximately 10 times higher in blacks living in the same areas

(Parkin & Muir, 1992). The differing rates of melanoma among different racial and ethnic groups point to the importance of genetic factor (Lew, Koh, & Sober, 1985).

2. Hair and eye color - red and blonde hair in childhood relative to dark hair were associated with increased risk of melanoma in most studies (Beral, Evans, Shaw, & Milton, 1983; Elwood et al., 1984; Graham & Graham-Tomasi, 1985; Hinds & Kolonel, 1983; Holman & Armstrong, 1984; Lew, Sober, Cook, & al, 1983).

3. Freckling - those who freckled readily were at consistently elevated melanoma risk relative to other individuals (Beral et al., 1983; Holman & Armstrong, 1984; Klepp & Magnus, 1979; R. Lew et al., 1983; MacKie & Aitchison, 1982).

4. Reaction to sun exposure - individuals who usually burn and are unable to tan are shown to be at a significantly higher risk of melanoma than those who tanned well (Beral et al., 1983; MacKie & Aitchison, 1982). Skin color (or degree of melanin) plays an important role in the determination of ultraviolet radiation (UVR) effects such as erythema or sunburn. Fair-skinned people require three to five times less UVR to induce erythema than do those with moderately pigmented skin, and up to 30 times less than darkly pigmented people (Wan, Jaenicke, & Parrish, 1983).

Most constitutional characteristics such as the above risk factors are interrelated. People that have fair skin, and light eyes and hair, probably are sun sensitive because they have less melanin, which protects their skin from the cumulative damage produced by UV radiation (National Cancer Institute, 1999a). The fact that these people suntan minimally, or not at all, and sunburn easily, presumably explains the high risk among lightly pigmented individuals.

Nevus Phenotype (Dysplastic, Nevus Type and Density)

Mole patterns, including type and number of moles, are an important risk factor for melanoma (Gallagher & McLean, 1995). Most moles, which are clusters of melanocytes, are benign lesions called common acquired nevi. This type of mole may undergo abnormal changes, and become a melanoma.

Alternatively, some melanomas arise in a skin site where there was not a preexisting mole. A persistently changed or changing mole, particularly in an adult, may be an important risk factor for the development of melanoma (Rhodes, Weinstock, Fitzpatrick, & al, 1987). Dysplastic nevi identify individuals at increased risk of melanoma, both in the familial and nonfamilial setting. Dysplastic nevi are especially important in familial melanoma. The risk of melanoma is highest for members of melanoma-prone families who have dysplastic nevi and who have already had a melanoma; they are at exceedingly high risk of developing additional primary melanomas (Tucker & Bale, 1988). Members of melanoma-prone families with dysplastic nevi but no personal history of melanoma are also at greatly increased risk of melanoma. The risk of melanoma among persons with dysplastic nevi but no family history of either melanoma or dysplastic nevi is increased, but is not nearly so high as in those with a family history of melanoma.

Between 2% and 4% of white adults may have at least one dysplastic nevus; the occurrence of dysplastic nevi in black African-Americans and Asians is much less (Rigel & Friedman, 1985). The risk for malignant change in a dysplastic nevus is significant. Clinical criteria for classifying a nevus as dysplastic include poorly defined

borders, irregular pigmentation, or a fried-egg pattern. Lesions are usually 5 millimeters in diameter or larger, but may be smaller.

Acquired nevi may develop in areas seldom exposed to direct sunlight - such as the buttocks or inside of the thighs - and these also carry a risk for developing melanoma or may be markers for melanoma risk. An acquired nevus does not necessarily have to be exposed to ultraviolet radiation to undergo malignant change.

Giant congenital nevi, which are present at birth or develop within the first year of life, are a risk factor for melanoma (Rhodes et al., 1987). The size of the mole appears to be an important risk factor for melanoma (Rigel & Friedman, 1985). However, even small congenital nevi have a significant melanoma risk. Moles known as giant congenital nevi have substantial risk for malignant change—about 15% over a lifetime (American Academy of Dermatology, 2000). The risk of melanoma associated with small congenital nevi is more controversial.

Previous Melanoma

Individuals who have already had one melanoma also have increased risk of developing additional primary melanomas (Evans et al., 1988; MacKie, Freudenberger et al., 1989; Rhodes et al., 1987; Tucker & Bale, 1988). At least 5% of persons who have had one melanoma will develop a subsequent, independent melanoma (Rigel & Friedman, 1985).

Family History of Melanoma

People with a family history of melanoma, even without dysplastic nevi, have increased risk (National Cancer Institute, 1999a). Approximately 8% to 12% of

malignant melanoma cases occur in individuals with a familial predisposition (Goldstein & Tucker, 1995).

Immunosuppression

Melanoma has been observed to occur more frequently in patients whose immune system has been rendered deficient by disease or by post-transplantation drugs. Diseases that suppress the immune system (and may have an increased risk for melanoma) include Hodgkin's disease (Tucker & Bale, 1988) and Acquired Immunodeficiency Syndrome (American Academy of Dermatology, 2000). Immunosuppressive drugs that are given to retard or prevent organ rejection after transplantation has been associated with an increased incidence of melanoma in transplant patients (American Academy of Dermatology, 2000; Barrett, First, Aron, & Penn, 1993; Bouwes Bavinck et al., 1996; Penn, 1977).

Xeroderma Pigmentosum

Individuals with xeroderma pigmentosum, a rare hereditary skin disease, lack an enzyme that normally repairs cellular DNA damaged by UVR and face increased risk of both melanoma and nonmelanoma skin cancers (Marzulli & Maibach, 1996). Certain hereditary conditions such as the extremely rare xeroderma pigmentosum are associated with a markedly increased risk for melanoma.

Prior History of Other Cancers

Prior history of nonmelanoma skin cancers was found to be associated with increase risk of subsequent development of melanoma (Holman & Armstrong, 1984; Marghoob et al., 1995). In addition, significantly elevated risks of melanoma are seen after brain and breast cancer (Tucker & Bale, 1988).

Socioeconomic Status

Potential relationships between socioeconomic status and melanoma incidence and mortality have been analyzed in several epidemiologic studies. Variables used to reflect socioeconomic status have included occupational groups (e.g., professional vs. laborer), types of work (e.g., indoor office vs. outdoor), and other indicators such as education and income. The studies have not produced a clear understanding of the relationship of melanoma to socioeconomic status. Several epidemiologic studies have indicated that melanoma incidence is positively related to socioeconomic status (Cooke, Skegg, & Fraser, 1984; Faggiano, Partanen, Kogevinas, & Boffetta, 1997; Gallagher et al., 1987; Lee & Strickland, 1980; MacKie & Aitchison, 1982). While some studies have shown that outdoor workers do not have an elevated risk of melanoma compared to office workers (Cooke et al., 1984; MacKie & Hunter, 1982) other studies have indicated that outdoor workers have slightly elevated melanoma risks for normally uncovered parts of the body such as the face and neck (Beral & Robinson, 1981; Vagero, Ringback, & Kiviranta, 1986). Professional and administrative type office workers, but not other indoor workers, have been shown to be at elevated melanoma risk compared to outdoor workers (Holman, Mulroney, & Armstrong, 1980; Lee & Strickland, 1980) and to have an elevated risk of melanoma on normally covered parts of the body (Beral & Robinson, 1981; Vagero et al., 1986). In the most recent study by Harrison et al (Harrison, Haque, Roseman, & Soong, 1998), they suggested that education over socioeconomic status was explaining the relationship with socio-economic status and melanoma.

Less Significant Factors

Other factors that have been studied and generally found less related to risk include: alcohol, caffeine, tobacco, hair dyes, pesticides, marital status, and parity (Longstreth, 1987). Melanoma has been found to be unrelated to tobacco smoking (Merimsky & Inbar, 1998; Osterlind, 1990). In a population-based case-controlled study including 474 cases with cutaneous malignant melanoma and 926 controls, the risk factors for developing melanoma were assessed. Sun exposure was strongly associated with increased risk for melanoma, but dietary factors, bathing habits, alcohol intake, and tobacco smoking were not related to risk of melanoma. While the relative risk of the never smokers was 1.0, the risk of the ex-smokers and the current smokers ranged between 0.8 to 1.1. The route of tobacco consumption (pipe, cigarettes, cigars or cigarillos) and the amount of tobacco smoked per day did not affect the risk of developing melanoma. Similarly, no occupational hazards, apart from UVR exposures, have been significantly identified to show a consistent risk for melanoma.

Pharmaceuticals

Oral contraceptives (OCs). Based on conclusions from several studies, Beral et al (1984) stated that while most studies reported weak or no associations of melanoma to ever-use of OCs, the five studies examining data on prolonged OC use found some increased risks (though not always statistically significant) associated with long-term pill use. However, in a more recent study by Grin et al (Grin, Driscoll, & Grant-Kels, 1998), exposure to OCs did not appear to increase the risk of melanoma.

Psoralen and ultraviolet A radiation (PUVA) therapy. As mentioned previously, the use of artificial ultraviolet sources along with oral psoralens to treat

some dermatologic diseases have been reported as a risk factor for melanoma. Psoralens are used for their photosensitive properties in this type of therapy. Epidemiologic studies of patients on PUVA have shown a dose-dependent increase in the incidence of NMSC, especially squamous cell carcinoma (Gupta & Anderson, 1987). Cases of melanoma have been reported in patients treated with PUVA, but in one study, rates were not much higher in these patients than would be expected in the population (Gupta & Anderson, 1987). A more recent study has shown that the therapy is associated with an increased risk (Wang et al., 2001).

Other photosensitizers. In January 2000, the FDA published draft guidance for industry on Photosafety Testing (FDA/Center for Drug Evaluation and Research, 2000). The FDA believes people who use photosensitizing compounds may be at greater risk than the general public. Data from animals and humans suggest that at least some photosensitizers enhance UV associated skin carcinogenesis.

Robust data in humans evaluating the relationship of exposure to orally ingested photosensitizing drugs, other than psoralen, to the risk of melanoma are not available. The fluoroquinolones have been demonstrated to be photoirritants and photochemical carcinogens in hairless mice. The published experimental experience for quinolones in animals provides a basis for a photocarcinogenic risk. Klecak (Klecak, Urbach, & Urwyler, 1997) performed studies that demonstrated that photosensitizing quinolones chronically administered increased the risk of skin tumor development and that the two most photosensitizing quinolones had the highest associated risks. Another study by Johnson et al (Johnson, Gibbs, & Ferguson, 1997) demonstrated that a limited number of high dose exposure to UV and quinolones can increase cancer risk in animals. Many

investigators believe that fluoroquinolone effects are mediated by reactive intermediates (Martinez et al., 1998) produced by UV- activation, but the exact mechanism by which the fluoroquinolones exert photoirritation in animals and humans and photochemical carcinogenicity in animals is currently being studied.

Acute photoirritation reactions that occur after use of photosensitizing compounds usually resemble an exaggerated sunburn. However, these reactions may range from a mild erythema to blistering of skin with sloughing. A relatively small percentage of the population may show clinical symptoms of photosensitization, a much larger percentage may have subclinical effects (FDA/Center for Drug Evaluation and Research, 2000).

Many compounds cause photosensitive reactions and some are listed in Appendix C. Tetracyclines are a widely used compound with a long history of photosensitive reactions dating back to their introduction in the 1950's. A literature search revealed no epidemiologic or current animal studies addressing their carcinogenic risks for melanoma.

The Exposure of Interest: Tetracyclines

History

The tetracyclines were discovered as the result of the systematic screening of soil samples from many parts of the world, for microorganisms capable of producing potentially useful chemotherapeutic agent. Mycologist B.M. Duggar, while working on Actinomyctales samples in 1944, noted that one of the cultures form a colony that produced an unusually broad antimicrobial spectrum of activity (Duggar, 1948). He identified the new species *Streptomyces aureofaciens* and named its product

Aureomycin (chlortetracycline hydrochloride) (Finland, 1974). Lloyd H. Conover invented tetracycline in 1948 by chemically altering Duggar's antibiotics. Within three years they became the most prescribed broad-spectrum antibiotic in the United States with over 250 tons produced in the United States (Goodman & Gilman, 1955).

The classical tetracyclines were derived from the *Streptomyces spp.*, but newer derivatives such as Doxycycline, approved by the FDA in 1967 and minocycline, approved in 1972, are semisynthetic derivatives (Vassileva, Mateev, & Parish, 1998). Tetracyclines are grouped according to their dosage and frequency of oral administration. Group 1 includes older derivatives such as chlortetracycline (not used much), oxytetracycline, and tetracycline. Group 2 includes demeclocycline and methacycline. Group 3 includes newer drugs such as doxycycline and minocycline.

Table 2.

Groups of Tetracyclines based on dosing interval.

Group 1 (Short Acting)	**Group 2 (Intermediate Acting)**	**Group 3 (Long Acting)**
Tetracycline	Demeclocycline	Doxycycline
Oxytetracycline	Methacycline	Minocycline

Mechanism of Action

Tetracyclines inhibit protein synthesis and are mainly bacteriostatic against most organisms. In high concentrations, however, tetracyclines can be bactericidal. Bacteriostatic action appears to be a result of reversible binding to ribosomal units of susceptible organisms and inhibition of protein synthesis. Tetracyclines gain access to

the ribosome after passive diffusion through channels in the bacterial membrane. Tetracyclines bind reversibly to the small subunits of bacterial (and eukaryotic) ribosomes where they interfere with binding of charged-tRNA to the "Acceptor" site. Bacterial protein synthesis is inhibited, which ultimately accounts for the antibacterial action. Tetracyclines can also inhibit protein synthesis in the host, but are less likely to reach the concentration required because eukaryotic cells do not have a tetracycline uptake mechanism.

Tetracyclines are primarily used by oral administration, but topical, intra-muscular, and intravenous forms exist (Reents, 1994).

Clinical Applications

Because tetracyclines inhibit bacteria, rickettsia, and chlamydia, they are true "broad-spectrum" antibiotics. The list of diseases for which tetracyclines can be used is long. Their applications are broader than many antibacterials. Appendix B is a table taken from the American Society of Health-System Pharmacists (AHFSDI, 2001) that gives the current FDA labeled and unlabeled uses of tetracyclines.

Adverse Reactions

Tetracyclines are generally regarded as relatively non-toxic, but they produce a fairly large number of adverse effects, some of which can be life threatening under the right circumstances. Appendix B gives some adverse reactions to tetracyclines. This study focuses on the photosensitive reactions caused by the tetracyclines.

Photosensitivity

Photosensitivity is a relatively general term used to describe any cutaneous reactions to light (UVR or visual). Photosensitivity reactions may be more specifically

categorized as phototoxic or photoallergic in nature. Sunburn is the most frequently occurring phototoxic reaction, requiring only the interaction of ultraviolet light with skin (Marzulli & Maibach, 1996).

Phototoxicity is used to describe all nonimmunologic light induced toxic skin reactions and encompasses sunburn and reactions caused by chemical photosensitizers, such as the tetracyclines. Clinical features of phototoxicity are not unique or particularly characteristic. Histopathological findings in phototoxic dermatitis due to chemical photosensitizers are identical to those in sunburn (Harber & Bickers, 1989). Patients are aware of sunburn that appears to be more severe than usual or is "exaggerated" (Harber & Bickers, 1989). These reactions are typically manifested by a delayed erythema and edema, followed by hyperpigmentation and desquamation.

Tetracyclines serve as excellent examples of the phototoxic hazards of chemical agents. The precise mechanisms of tetracycline phototoxicity are not fully understood. In vitro studies have shown that they are dependent on oxygen and complement. The chromophores in the skin are cell membranes, ribosomal proteins, and DNA. Two major types of damage induced in DNA molecules by the photosensitizing activity of tetracycline derivatives are proposed; these are alteration of guanine residues and breakage of the sugar-phosphate backbone (Layton & Cunliffe, 1993).

When tetracyclines were first introduced there were no warnings about their potential for photosensitization. These warnings have been added to their labels only after adverse reactions resulted not during phase 2 or 3 clinical trials, but during widespread clinical use of the products. Of the tetracyclines in current common use, reactions are seen with use of all types in this class, but Vassileva et al (1998) reported

reactions in 20% of those taking doxycycline, 7% of those taking methacycline, and very infrequently with use of minocycline.

Photosensitivity, if apparent, can appear within minutes of taking the tetracycline if the patient is exposed to a delimited range of the electromagnetic spectrum that includes visible light and UV radiation (Vassileva et al., 1998). Ultraviolet (200-400 nm) and visible (400-800 nm) lights, to which the skin is continually exposed, are produced by the sun and artificial sources, such as tanning beds and fluorescent lamps. Common clinical manifestations of tetracycline photosensitivity include phototoxic reaction manifested as sunburn, sometimes with blistering, papular eruption, or both (Vassileva et al., 1998). Warning signs, including tingling and burning of the hands, feet, and nose, may indicate latent photosensitivity. If the drug is discontinued, symptoms are usually alleviated within 1-2 days. Sunscreens seem to provide only limited protection, and a severe response may necessitate treatment with corticosteroids or antihistamines. Rashes and discolored nails have been reported.

The damage is generally thought to be due to excessive oxidation/ peroxidation of cellular components. Factors influencing the likelihood of reactions include: a) amount of drug/ metabolites in the skin; b) intensity and spectrum of sunlight exposure; and c) thickness of keratin layer and melanin content of skin.

Figure 11 shows the proposed mechanism of a phototoxic reaction. A chromophore is a molecule or substance that absorbs the light. In the mechanism of a tetracycline phototoxic reaction the chromophore is the drug itself once absorbed in the bloodstream.

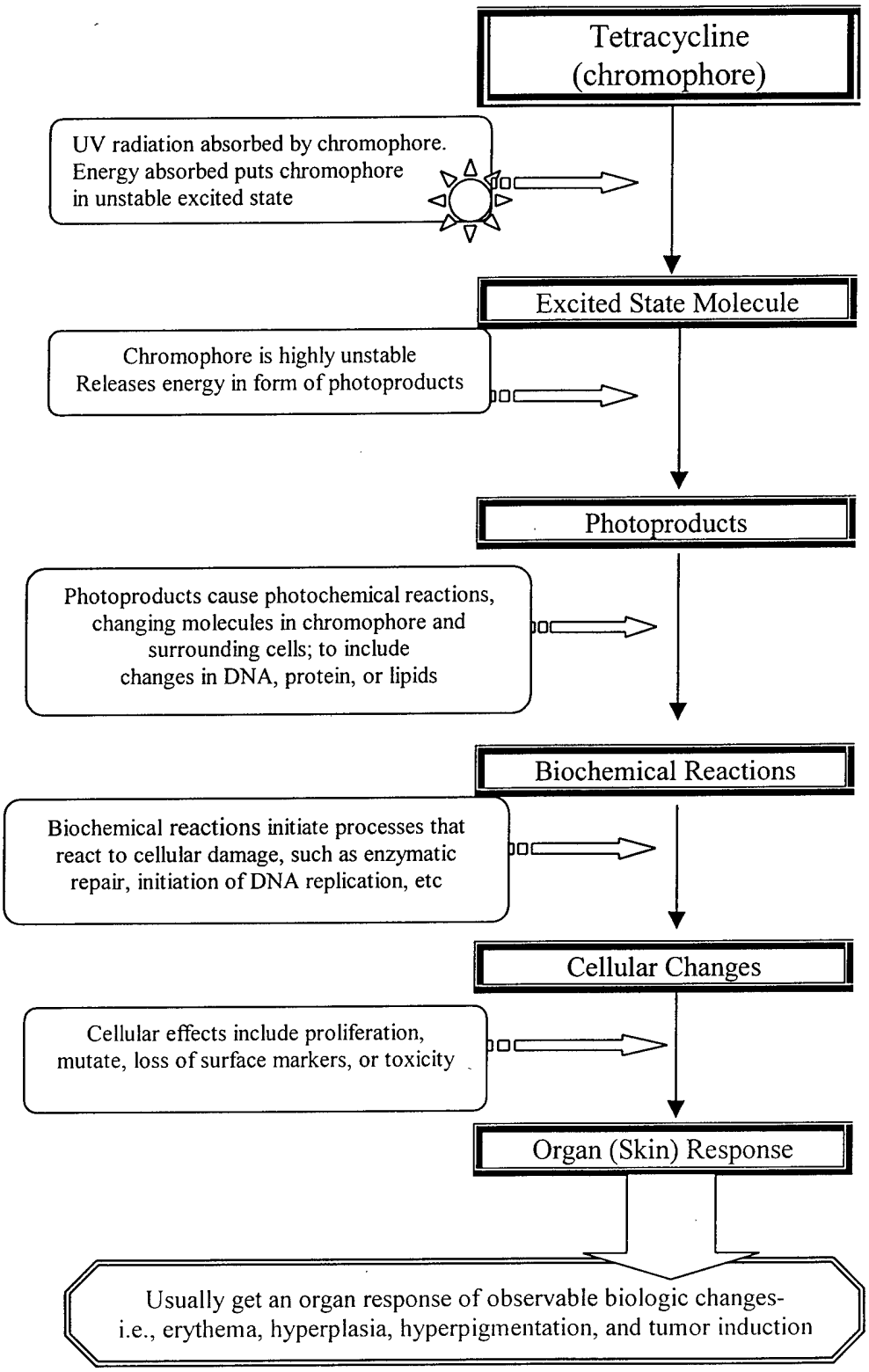


Figure 11. Proposed processes in a phototoxic reaction.

Mutagenicity and Carcinogenicity

Although no studies were found on association of tetracyclines and skin cancers, some studies have reported that tetracycline antibiotics (tetracycline, oxytetracycline) have demonstrated mutagenic potential in vitro mammalian cell (e.g., mouse lymphoma, Chinese hamster lung cell) assays (AHFSDI, 2001). Long-term dietary administration of minocycline has resulted in evidence of thyroid tumors in rats, and adrenal and pituitary tumors have been reported in rats receiving oxytetracycline. However, in studies conducted in mice and rats, tetracycline hydrochloride did not demonstrate this type of carcinogenicity.

Prevalence of Use/Public Health Impact

Although many medications can cause photosensitive reactions, the long-standing availability and widespread use of tetracyclines could impact the health of many people if the drug was found to be associated with a greater risk for cancers such as melanoma. Tetracyclines have been around since the 1950's and are having a resurgence of popularity. They continue to be used extensively today, with newer uses increasing the number of prescriptions filled. Up to 9 million prescriptions for tetracyclines were filled annually just in non-hospital pharmacies alone; as reported by the Johns Hopkins University of Medicine Division of Infectious Diseases (Johns Hopkins University Division of Infectious Diseases, 2000). They listed tetracyclines on their Top 7 Antibiotics in Prescriptions list from 1992-1996.

Similarly, in 1996 in the United Kingdom, 28 million minocycline tablets were taken. An estimated 65% of minocycline prescriptions were for acne (Unknown, 1998).

Rationale and Hypothesis of the Melanoma and Tetracycline Relationship

Research suggests melanoma skin cancers are caused by intermittent periods of intense sun - the kind that causes sunburn - rather than repeated exposure over time like other forms of skin cancer(American Cancer Society, 1999b). The physiological theory, proposed by Dr. Gilchrest et al (1997), describes how UV-damaged melanocytes mutate and divide as a result of sunburn increasing the risk of melanoma with each sunburn. A similar theory can support a tetracycline-induced sunburn-like reaction which could also increase risk of melanoma. Harber and Bickers (1989) acknowledged that the histopathological findings in drug induced phototoxic dermatitis are identical to those in sunburn.

It is well established that ingesting photosensitizing, phototoxic drugs, like tetracyclines, greatly increases the degree of erythema an individual experiences with an exposure to natural or artificial ultraviolet light. The extent to which this photo-sensitive reaction may increase cancer risk in humans is not established.

The Federal Drug Administration (FDA) Center for Food and Drug Research recognizes the potential for photocarcinogenicity of photosensitizing drugs. Data from studies in animals and humans support the theory that at least some photosensitizers may enhance UV-associated skin carcinogenesis (FDA/Center for Drug Evaluation and Research, 2000). The FDA admits to a historical lack of research and the need for further study of this relationship because the majority of systemically administered drugs have not undergone specific controlled testing to elucidate their potential for enhancing UV-mediated carcinogenic effects on the skin. Their draft guidance

recommended that once a systemically or dermally administered drug has been identified as a photosensitizer in animal or human testing, one should consider the drug's potential to increase UV-associated skin cancer risk.

Tetracycline antibiotics serve as excellent examples of photosensitive properties that have been identified through human testing. Their phototoxic potential appears to be elicited by ultraviolet A (UVA) rays, with some data suggesting augmentation with UVB (Vassileva et al., 1998). Since UVA light easily penetrates the entire epidermis, phototoxic reactions could easily occur at the level of the melanocytes (Arbesman, 1999). These reactions can produce free radicals, leading to cellular injury of the skin including the melanocytes. This injury to the melanocytes may lead to the initiation and/or promotion of melanoma (Kraus, 1996). This type of cellular injury usually manifests clinically as a sunburn-like reaction; however, many individuals using tetracyclines do not detect any physical signs of photosensitivity. Photosensitization could still be producing free radicals on a microscopic level resulting in subclinical damage to melanocytes (FDA/Center for Drug Evaluation and Research, 2000).

Many of the well-documented risk factors for developing melanoma, such as fair skin, history of sun exposure and sunburns, can be consistent with the hypothesis that tetracyclines are a risk factor for melanoma. The recent rise in melanoma in a worldwide distribution (Koh, Kligler, & Lew, 1990) since the 1950s and then the dramatic increase after the 1970s correlates well with the timing of the initial use of tetracycline antibiotics and then their popularity in the treatment of acne. This hypothesis could also explain the findings that there is a higher incidence of melanoma in indoor workers (Koh et al., 1990), those of higher socioeconomic status (Gallagher et

al., 1987; Koh et al., 1990), and the rise in younger individuals (Osterlind & Jensen, 1987); if the use of tetracycline antibiotics is greater in these groups. The use of antibiotics may be higher among the wealthier because of better access to healthcare. Treatment for adolescent acne may increase the use of these antibiotics in younger individuals. The higher incidence of melanoma (Koh et al., 1990) in sunnier areas is consistent with this hypothesis since a greater exposure to sunlight along with photosensitization could lead to a higher incidence of melanoma. One possible explanation for areas throughout the world where the melanoma risk is not associated with the latitude gradient relationship (Koh et al., 1990) could be that individuals in certain areas may not use or require antibiotics as frequently as individuals in other areas. The racial differences in melanoma incidence may be explained by melanin protection, but also by the more infrequent use of tetracyclines (i.e. reduced availability, cost prohibitiveness, or not proned to acne).

The differences in types of melanomas may partially be explained by the use of tetracycline. Areas of pigmentation associated with the use of tetracyclines follow some of the patterns of development of the more uncommon melanomas - hyperpigmentation of nailbeds, sclera, hair, thyroid, and tongue (Karofsky & Williams, 1995).

Current widespread use and long-term therapies (such as with the treatment of acne), in conjunction with sun exposure, results in frequent opportunities for photosensitization (sunburn-like reactions) in individuals to occur (Stern, 1998). If this hypothesis is correct, and a portion of the recent increase in incidence of melanoma can

be attributable to the use of tetracycline antibiotics, then a reduction in the number of new melanomas may be attained with the discontinuation or limited use of these drugs.

CHAPTER THREE: PROCEDURES AND METHODS

Study Overview

This study is a case control study exploring the relationship between tetracyclines and melanomas using white male patients seen at the James A. Haley Veterans Hospital from January 1994 through December 2000. It utilizes the linking of four computerized databases at the James A. Haley Veteran's Hospital - pathology, pharmacy, laboratory, and administrative - for patient information.

Linking records can provide a quick and useful means for testing hypotheses before engaging in more definitive, costly studies. Studies such as this one, which tests an association between drug therapies and disease outcome, can help identify potential etiological risk factors. These risk factors can then be included in other studies for further analysis.

For the study of tetracyclines and melanoma, this is an ideal population. This hospital has had historically high incidence rates of melanoma, a steady prescription rate of to tetracycline drugs for the last four years, and a fairly stable patient population with reasonably good access to subsidized healthcare.

The number of melanomas diagnosed in this population is three times (in some age categories) what would be expected, based on rates reported by SEER. Using SEER rates as a standard population rate, observed rates in the James A. Haley Veterans Hospital population were compared to those from the 1994-1998 SEER

report. Standardized morbidity ratios for each age category were calculated along with their 95% confidence intervals. The results are listed in Table 3.

Table 3.

Standardized Morbidity Ratios for Source Population using SEER reported data from 1994-1998 as a standard.

Age	SMR	95 % Confidence Limits
<46	2.9702	0.7699, 5.1706
46-55	1.7850	0.8147, 2.7554
56-65	3.7031	2.3995, 5.0067
66-75	3.7930	2.8709, 4.7151
76-85	2.8547	2.0712, 3.6382
>85	2.8914	0.5778, 5.2050

Note: Calculation includes all males in source population.

Ethical Concerns

Approval for the study was obtained from both the James A. Haley Veteran's Hospital and the University of South Florida Institutional Review Boards. Patient confidentiality was maintained through the use of only scrambled social security numbers links with no distinguishable patient identifiers.

Source Population

All subjects for the study were selected from computerized patient administrative or pathology records maintained at the James A. Haley Veterans' Administration Hospital, located in Tampa, Florida. The hospital is a 431-bed Level III tertiary care teaching hospital with a diverse patient mix serving 350,000 veterans in

central Florida. The hospital consists of 150 medicine, 96 surgery, 75 psychiatry, 60 spinal cord injury, 42 rehabilitation medicine and 8 neurology beds. A 180-bed Nursing Home Care Unit is attached to the hospital. Complete ranges of inpatient and outpatient services are provided in all specialties and subspecialties of medicine, surgery, psychiatry, radiology and pathology. It is one of the busiest centers in the VA system, with over 500,000 outpatient visits with almost 80,000 individual patient records last year.

Subject Selection

All study subjects were selected from either the hospital's computerized pathology databases or centralized administrative database.

Cases

Only incident cases were eligible for the study. Incident cases were those patients in the James A. Haley Veterans' Hospital pathology database with an initial diagnosis of melanoma. Computerized pathology databases began in 1992, with reliable data collection estimated to start in 1994.

Initially, 327 cases were found during the years 1994-2000 in the pathology database. However, 40 of these were tissue sample referrals to the James A. Haley pathology department from other VA clinics and hospitals in Florida. Because they were not patients, they had no hospital administrative records for demographic data. These cases were not considered part of the source population and thus excluded from eligibility. After elimination of non-patient cases, four cases were selected as controls during randomization and were not included in the analysis due to potential problems with independence. This totaled 283 cases for the study.

Controls

Controls were randomly selected from the James A. Haley computerized administrative database. A subject was eligible to be a control if they had a recorded history of a patient visit from 1994 through 2000 and no prior history of melanoma. Over the 7-year study period, there were 310,357 individual patients available for study selection. Of these, 6163 records had a gender classification of "UNKNOWN" and were omitted as a potential study participant. Of the 304,194 patient visits with known gender, 264,783 (87%) were males and 39,411 (13%) were females. These patients had over 423,000 documented patient visits. The distribution of age of the source population was as follows: a) 17.5% were less than age 45, b) 22.5% were 45-55 years old, c) 17% were from 56-65 years of age, d) 22.7% were 66-75, f) 18.3% were from 75-85 years old, and g) 1.9% were older than 85.

Eligible controls were initially separated based on the year of visit to the hospital (both inpatient and outpatient visits were counted). Once separated by date of visit, using SAS® statistical software (ranuni function), patient visit records were randomly assigned numbers for inclusion into the study. Controls were sorted based on randomized numbers. A control was considered for the study if it matched the case (original 327 cases) on year of birth and gender, and who was seen as a patient the same year the case was diagnosed but had not been diagnosed with melanoma of the skin that year or any year prior to inclusion into the study. Six controls were then selected based on meeting the case birth year, gender and year diagnosed with melanoma. It was possible that a control selected in previous years could become a case or control during later years. Four study subjects were selected as both cases and

controls and were not included in the analyses due to independence problems. No duplicate controls were encountered. The number of controls selected initially for the study was 1884.

Figure 12 visually displays the subject selection and risk year groups.

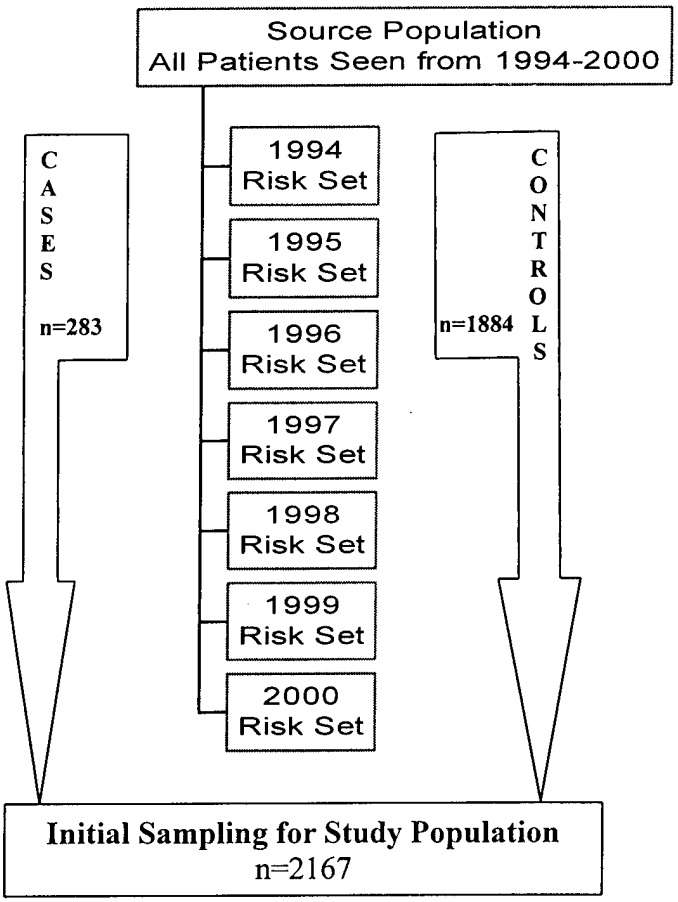


Figure 12. Case control selection risk sets for study population.

Note: Risk sets were defined by year of case diagnosis. Controls were patients "at risk" or seen as a patient the same year case was diagnosed, and same age and gender as the case.

Matching

Matching on gender and age was performed to ensure comparable distribution of gender and age groups among the controls in the original selection of controls. This matching was not preserved when the study was limited to White and Hispanic White men.

Sample Size and Power

Sample size and power calculations were performed prior to start of the study. The exposure estimation before the study was based on the number of individual patient prescriptions of tetracycline class of antibiotics filled during 1997 through 1999. With the denominator as the total number of active patient records during these years, a very stable average of a 2.12 % exposure rate was estimated. With 327 cases available initially, and this estimated 2.12% exposure rate, an odds ratio (OR) of 3.0 could be detected with a power of 80% and 95% confidence. There were not enough cases to detect an OR less than 3.0 with 80% power and 95% confidence. An alpha level of .05 level was considered as significant in all analyses.

When cases were found to be only referral slides to the pathology department, and were eliminated, the original power calculations could not be met. Also, because ethnicity has shown to be such a strong risk factor (whites 10 times higher rates than blacks) for melanoma and 25% of ethnicities were missing, the study was eventually limited to white and Hispanic white men to retain validity. The final study population consisted of a total of 1271 white men, with 208 controls and 1063 controls. Five of these subjects had missing information on branch of service, so the total number of subjects available for full analyses was 1266. The exposure rate in the controls was

5.83% and the in the cases, 10.53%. With the sample size now predetermined, the power of the study was calculated using Epi Info StatCalc. Based on the total sample size of 1271, consisting of 209 cases and 1035 controls, the power of the study to detect an odds ratio of 2.01 was 66% with an alpha level of .05 (95% confidence).

Missing Data and Exclusions

After initial exclusion of patients with missing or unknown gender and year of birth, records of subjects who had missing data on ethnicity were scrutinized because ethnicity has consistently been shown to be a strong risk factor for melanoma. Initially, 798 subjects had missing entries or entries of "UNKNOWN" in the ethnicity data field. After further review of computerized medical records for documentation of patient ethnicity by a provider, 270 subjects' ethnicities were ascertained. This still left 528 subjects without a known ethnicity. A comparison of risk for melanoma between the subjects with missing or unknown ethnicities to those with known ethnicities revealed the subjects with unknown ethnicities had a two times higher risk of melanoma than those with known ethnicities, after adjusting for baseline characteristics. After this revelation, it was decided that the analyses would be restricted to those subjects with known White or Hispanic White ethnicities to preserve validity. This exclusion minimized the number of women available for the study, so the study population was finally restricted to men only. The analyses included only White and Hispanic White men and their records had only 5 missing data field entries in the branch of service variable.

Exposure Estimation

Estimates of exposure to tetracyclines were based on data collected from the James A. Haley pharmacy database. Computerized collection of pharmacy records was initiated in 1992. The groups of tetracyclines found in the database for this study population included tetracycline 250 mg, tetracycline 500 mg, doxycycline 50 mg, doxycycline 100 mg, minocycline 100mg and demeclocycline 100mg.

An exposure was defined as a prescription filled by the pharmacy that was a member of the tetracycline class of antibiotics at least one year before the subject was diagnosed as a case. Control pharmacy histories were censored at the index case's diagnosis date to ensure case control exposure time coincided.

Baseline Characteristics of Study Population

Available demographic information obtained from the administrative database included gender, date of birth, marital status, branch of service history, ethnicity, combat history and prisoner of war history. Human immunodeficiency virus status was obtained from the laboratory database. Smoking status and additional ethnicity information were gleaned from individual computerized patient medical records. Exposure information to other select photosensitizing drugs was abstracted from the pharmacy database.

The baseline characteristics available have been previously identified in other studies to have associations with melanoma:

1. Age - Increasing age is related to increasing incidence of melanoma (Dennis, 1999).

2. Marital Status - Marital status may be associated with the diagnosis of melanoma. Married males could have earlier diagnoses (possibly because of partner identification of lesions), thus are thought to have a higher incidence but probably a lower mortality as compared to nonmarried individuals. One might expect a higher incidence of melanomas in married persons because of better detection of lesions.

3. Branch of Service - Service is thought to be a risk factor due to the potential for different exposures to sun and potential carcinogens among troops in the different services. Branch of service may be associated with sun exposure because field troops may be more exposed to the sun and/ or carcinogens than those who work indoors. However, persons with indoor occupations have been shown to be at increased risk for melanoma and pilots may have higher UV doses than most because of exposures incurred at high altitudes in the cockpit. The Air Force was conceived in 1947, so the Army may be over represented in this population.

4. Prisoner of War - One recent study found that Prisoners of War in the Pacific theater had a higher rate of mortality from melanomas than other veterans, possibly due to exposures to sun during young adulthood (Page, Whiteman, & Murphy, 2000).

5. Combat Duty - No published studies were found to show that veterans who served in combat situations had a higher rate of melanomas, but previous unpublished studies (Rae, 2000) have shown combat duty to be related to the increase incidence of nonmelanoma cancers.

6. HIV Status - Immunosuppression has been shown to increase the risk of melanoma. The American Association of Dermatologists (2000) lists Human Immunodeficiency Virus infection as a risk factor for melanoma.

7. Risk Year - The year the patient was diagnosed as a case or selected as a control may be associated with the incidence of melanoma due to diagnoses biases among physicians finding the lesions, and among pathologists correctly identifying the malignant cells. Over a 7-year time frame, changes or increases in medical personnel can bias the incidence of melanoma. Improvements in technologies or patient education may also affect incidence of disease. Exposure rates may increase over the years as new therapies are developed, though in the source population the estimated prescription rates for tetracyclines remained stable during the study period.

8. Smoking History - Smoking has been linked to many cancers but has not consistently been shown to be associated with melanoma (Longstreth, 1987). Because of smoking's detrimental effects on the body's immune system one might expect to find an association.

9. Other known photosensitizers – Drugs such as flouroquinolones and psoralens have been show to be associated with an increased risk for skin cancers. The prescribed medications for this population were reviewed, and historically well-recognized photosensitizing drugs were grouped together into one variable (see Appendix C). It would be expected that the use of other photosensitizing drugs would affect a relationship between tetracyclines and melanoma if photosensitization were a mechanism for risk.

CHAPTER FOUR: PRESENTATION AND ANALYSIS OF DATA

Variables

Dependent

The main outcome variable, melanoma, was defined for analysis as 0, or no diagnosis of melanoma and 1, or initial diagnosis of melanoma by James A. Haley Pathology Department.

Independent

The exposure variable, tetracycline, was treated in successive analyses treated as a binomial, categorized, and continuous variable in the analysis. The distributions of the continuous forms of the exposure variable were examined using PROC UNIVARIATE in the SAS software package. Because of the broad range in total milligrams, the dose of the drug was initially redefined into number of pills. This was calculated by dividing the total milligrams of: a) doxycycline by 100, b) tetracycline by 250, c) minocycline by 100, and d) demeclocycline by 150. However, this number was still very small when trying to compare levels from a logistic regression model. For a better comparison of dosing, the drugs were again redefined based on their normal prescribed treatment regimens. This was calculated by dividing the number of pills prescribed by the number of pills normally recommended for one treatment regimen of the tetracycline group of drug. Doxycycline 100 mg pills are normally prescribed twice a day for 14 days thus this was the definition of the doxycycline dose

(#100mg pills/28= 1 doxycycline therapy). Tetracycline 250mg tablets are usually prescribed for 1 gram daily divided into 4 doses (#250mg pills/56=1 tetracycline therapy). Minocycline 100mg tablets course of therapy is 2 tablets daily for 7 days (#100mg pills/14=1 minocycline therapy). Demeclocycline 150mg pills are prescribed 4 times a day for 10 days (#150mg pills/40). When combining the drugs for a total dose variable, the drug doses were combined based on their treatment regimens. The formula was totdose = (doxy100/28) + (tetra250/56)+ (min100/14)+ (deme150/40). This translated into the usual course of therapies as follows: a) Doxycycline 100 mg tablets, two tablets daily for 14 days; b) Tetracycline 250 mg tablets, four tablets daily for 14 days; c) Minocycline 100 mg tablets, two tablets daily for seven days; and d) Demeclocycline 150 mg tablets, four tablets daily for 10 days.

The continuous variable for total pills taken, of any type of tetracycline drug, showed a highly skewed distribution that had two very notable outliers. However, logistic regression analysis does not require multivariate normality and adequately handles data with large sample sizes. The continuous variables of doxycycline and tetracycline type of tetracycline drugs were examined individually in the analysis to examine their specific contributions to the risk of melanoma. Seven subjects had taken both drugs.

Nine independent variables (refer to Table 5), besides the tetracycline exposure variables (Table 4), were available for inclusion as covariates in a logistic regression model. Eight were used in most of the analyses. Smoking was not used in many of the analyses because of the numerous missing data and the imprecise definition of nonsmoker.

Four variables were binomial (HIV Status, POW Status, Combat Duty, Other Photosensitizing Drugs), four contain greater than 2 categories (Risk Year, Marital Status, Service and Smoking) and AGE was the only continuous variable. Results from a univariate procedure revealed the AGE variable did not follow a normal distribution based on significance testing, but the plot showed a distribution that basically followed a normal distribution with no extreme outliers. These variables were included in all models to control for any effects they may have on the outcome of melanoma and on the melanoma tetracycline relationship.

Distributions

Exposure

The distribution of the exposure of interest differed between the cases and controls. The cases received almost twice the number of tetracycline prescriptions as the controls. The specific tetracycline drug was prescribed almost four times more to cases than controls. Table 4 shows these distributions of the exposure of interest, tetracyclines, between the cases and the controls.

Table 4.

Tetracycline Exposure Distribution Information for Study Subjects

Total No. in Study Population = 1271	**Cases (N=208)**	**Controls (N=1063)**
Tetracycline Exposure		
Total No. Subjects Receiving Tetracycline Prescriptions	23(11.06%)	62 (5.83%)
Drug Specific Exposure		
Tetracycline Only	10(4.81%)	13 (1.22%)
Doxycycline Only	13(6.25%)	38 (3.57%)
Tetracycline & Doxycycline	0	7 (.659%)
Minocycline	0	2 (.188%)
Tetracycline & Minocycline	0	1 (.094%)
Demeclocycline	0	1 (.094%)
Mean Dose Prescribed in milligrams (sd)		
Doxycycline	1522.12 (+/-7357.97)	814.4873 (+/- 6463.04)
Tetracycline	8355.77 (+/-48937.39)	2081.37 (+/-16441.79)

Note: +/- and (sd) = standard deviation. Dose = milligrams in pill multiplied by number of pills prescribed.

Baseline Characteristics

Even after limiting the study to just White and Hispanic White men, the mean age between cases and controls was similar. Most of the distributions between the cases and controls were comparable. Table 5 shows the baseline characteristics of the study population and their distributions between the cases and controls.

Table 5.

Distribution of Baseline Characteristics of Study Subjects.

Total No. in Study Population = 1271	**Cases (N=208)**	**Controls (N=1063)**
Age		
Mean Age (sd)	68.303 (+/-11.520)	68.130(+/- 11.342)
33 to 50 years	15(7.21%)	72 (6.77%)
51 to 70 years	92(44.23%)	486(45.72%)
71 and older	101(48.56%)	505(47.51%)
Other Known Photosensitizing Drugs		
Prescribed	75 (36.06%)	338 (31.80%)
Not Prescribed	133 (63.94%)	725 (68.20%)
Risk Set Year		
1994	20 (9.62%)	73 (6.87%)
1995	21(10.10%)	94 (8.84%)
1996	22(10.58%)	109 (10.25%)
1997	27 (12.98%)	129 (12.14%)
1998	41 (19.71%)	230 (21.64%)
1999	35 (16.83%)	194 (18.25%)
2000	42 (20.19%)	234 (22.01%)
Marital Status		
Never Married	10 (4.81%)	66 (6.21%)
Divorced	48(23.08%)	183 (17.22%)
Separated	6 (2.88%)	22 (2.07%)
Widow/Widower	19 (9.13%)	89 (8.37%)
Married	125(60.10%)	703 (66.13%)
Service		
Air Force	40 (19.32%)	162(15.30%)
Army	103 (49.76%)	627(59.21%)
Navy	47 (22.71%)	183 (17.28%)
Marine Corps	15 (7.25%)	79 (7.46%)
US Coast Guard or Merchant Marine	2(0.97%)	8 (0.76%)
Missing	1(0.48%)	4 (0.38%)
Known Prisoner of War		
Yes	4 (1.92%)	25 (2.35%)
No	204(98.08%)	1038(97.65%)

(table continues)

Table 5. *(continued)*

	Cases (n=208)	Controls (n=1063)
Participated in Combat Duty		
Yes	73 (35.10%)	393 (36.03%)
No	135 (64.90%)	670 (63.03%)
HIV status		
Laboratory seropositive	2 (0.96%)	7 (.66%)
No history of seropositivity	206 (99.04%)	1056 (99.34%)
Smoking Status		
Documented as a never smoker or a nonsmoker	96 (46.15%)	265(24.93%)
Past history of smoking	21 (11.00%)	83 (13.14%)
Current history of smoking	51 (24.52%)	270 (25.40%)
Unknown smoking history	40 (19.23%)	445 (41.86%)

Note: +/- and (sd) = standard deviation.

As evident in Table 5, there were significant differences in smoking histories, with 42% of the controls and 19% of cases with no documented smoking history. Also, 46% of the cases had a documented nonsmoker history as compared to 25% of the controls. Marital status frequencies were distributed differently than expected, with more cases never married or divorced than noncases, though not significantly overall. Although not significantly different at the $p>.05$ level, more cases were prescribed other selected known photosensitive drugs than the controls (36.06% vs. 31.80%, respectively). Fifty six percent of the subjects prescribed tetracyclines were prescribed another photosensitizing drug. The distribution between the cases and the controls for being prescribed tetracycline and another photosensitizer was 48% and 60% respectively.

Testing the Hypothesis

Potential Confounders, Associations and Correlations

Each variable, as listed in the tables, was analyzed for their association with the disease and exposure of interest. Age, a continuous variable, was categorized and subsequently all variables were examined for their associations with the exposure (categorized version of exposure to any tetracycline drug, 0=no 1=yes) and the disease (melanoma) using Mantel Haenszel tests for association. Potential confounding variables for the tetracycline-melanoma relationship were identified by frequency distributions and by tests for associations. The variables identified as weak potential confounders using this method were smoking status, exposure to other known photosensitizing drugs, and marital status.

No correlations between variables were identified using correlation tests (Appendix D, Table D1).

Primary Analysis of the Tetracycline and Melanoma Association

The primary analysis was directed at finding whether a significant difference in risk of melanoma exists between patients who received a tetracycline class of drug prior to diagnosis of melanoma versus those patients who did not receive tetracycline therapy prior to their inclusion into the study. The variables used included tetracycline exposure variables, baseline characteristics of the population and known exposure to other tetracyclines. In most of the analyses smoking was not included because of the missing information. Five patients were excluded from the all the full model analyses because of missing information. All risk analyses were performed through logistic regression techniques using SAS® statistical software.

Crude Analyses

Initially, a crude odds ratio was estimated for risk of melanoma if a subject was exposed to any one of the four types of tetracycline drugs (exposed=yes or no). A crude OR was calculated for the continuous form of the above variable (variable TOTDOSE) which represented the risk associated with one treatment regimen of any tetracycline type drug. The total number of subjects available for these analyses was 1271. Table 6 shows the results of these overall crude analyses.

Table 6.

Crude Analyses of the Risk of Melanoma with Use of Tetracyclines.

Model 1 Melanoma = Any type of Tetracyclines prescribed -Yes/No

Model 2 Melanoma = Total # of Tetracycline Type Drug Therapies prescribed - Continuous

Exposure	**Risk Comparison**	**Crude O.R.**	**95% C.I.**
Model 1 Total Tetracyclines	Ever vs. Never prescribed any tetracycline	2.007	1.213-3.321
Model 2 Tetracycline therapy	1 treatment regimen of any type of tetracycline	1.025	.995-1.055

Full Model Multivariate Logistic Regression

A main effects model was then fitted using all available information on the subjects, except smoking. Smoking was not included because of the number of subjects with missing data. The results of this model were based on 1266 subjects due to 5 subjects missing information on their branch of service. Table 7 shows the results of this model.

Table 7.

Multiple Risk Equation for Melanoma: Logistic Regression Model Relating Tetracycline Use and Baseline Characteristics to the Incidence of Melanoma.

Model

Melanoma = Total Tetracyclines + Other photosensitive drugs + Age + Marital Status + Branch of Service + Combat Duty + Risk Year Group + HIV Status + POW Status

Variable	**Risk Comparison**	**Odds Ratio**	**95% Confidence Interval**
Total Tetracyclines	0=No tetracycline drug prescribed 1=At least one of four tetracyclines prescribed	**2.064**	**1.228-3.467**
Other known photosensitizing drugs	0=not prescribed 1=prescribed	1.169	0.848-1.612
Age	1-year age difference	1.000	0.986-1.014
Marital Status	Married = Reference		
	Never Married vs. Married	0.871	0.431-1.710
	Divorced vs. Married	1.489	1.020-2.174
	Separated vs. Married	1.569	0.611-4.025
	Widow vs. Married	1.259	0.733-2.161
Branch of Service	Air Force = Reference		
	US Coast Guard or Merchant Seaman	0.947	0.190-4.724
	Marine Corps	0.754	0.387-1.470
	Navy	1.042	0.646-1.681
	Army	0.670	0.444-1.010
Combat Duty	0=No 1=Yes	0.962	0.697-1.328
Risk Year Group	1994 = Reference		
	1995	0.873	0.433-1.758
	1996	0.740	0.370-1.477
	1997	0.806	0.418-1.555
	1998	0.685	0.374-1.256
	1999	0.668	0.358-1.246
	2000	0.684	0.374-2.885

(table continues)

Table 7. *(continued)*

Variable	Risk Comparison	Odds Ratio	95% Confidence Interval
HIV status	0 = no history of seropositivity 1 = seropositivity documented	1.476	0.294-7.404
Prisoner of War	0 = No 1 = Yes	0.971	0.327-2.885

Note: References are 0 unless otherwise state. Model based on 1266 total subjects.

Other Analyses

Dose Response Relationship

The next research question to address is whether there is a dose response relationship between amount of tetracycline type drugs prescribed and risk of melanoma. To examine this relationship the number of treatment regimens by the logodds for the risk of melanoma was plotted (Figure 13).

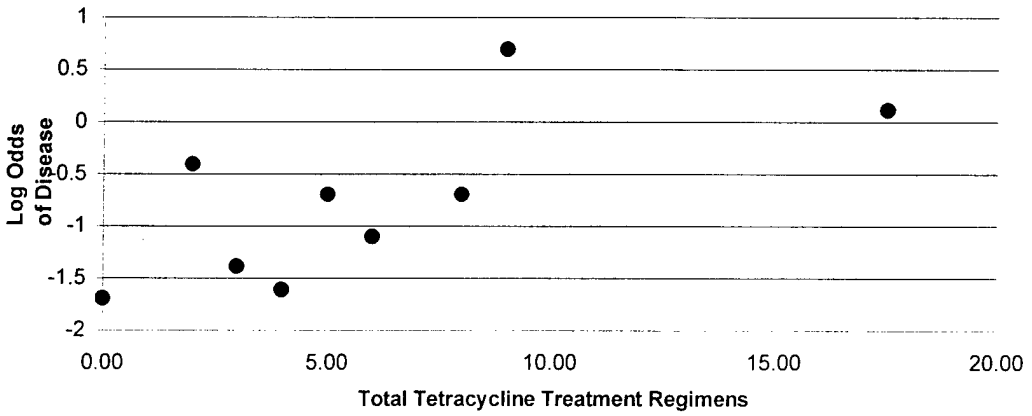


Figure 13. Plot of log odds of disease by number of treatment regimens prescribed.

The plot reveals a somewhat linear dose response relationship (until the highest dose) trend between the number of regimens of tetracycline type therapies and the risk of melanoma. To see if this was a significant trend, a Mantel Haenszel Chi Square test

for trend was performed on the logodds for melanoma by the number of treatment regimens prescribed. The results of this linear trend test were significant with a chi square of 3.6707 and p value of 0.0554.

Because the trend was significantly linear, and the data did not fit a normal distribution, a Spearman Correlation Coefficient test was performed with a Spearman correlation coefficient = .68620, p = .0412.

Next, plots of regimens of tetracycline 250 mg and doxycycline 100mg by the proportion diseased were created. Figures 14 and 15 visually show these plots.

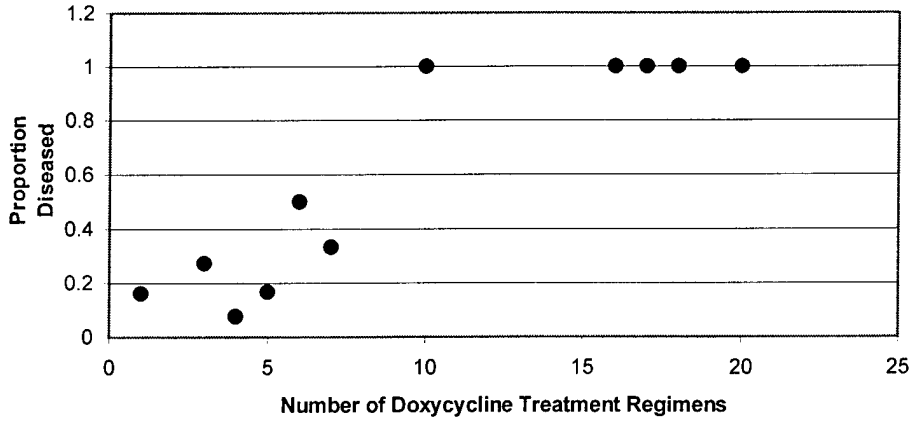


Figure 14. Plot of the proportion diseased by the number of treatment regimens of Doxycycline 100 mg prescribed.

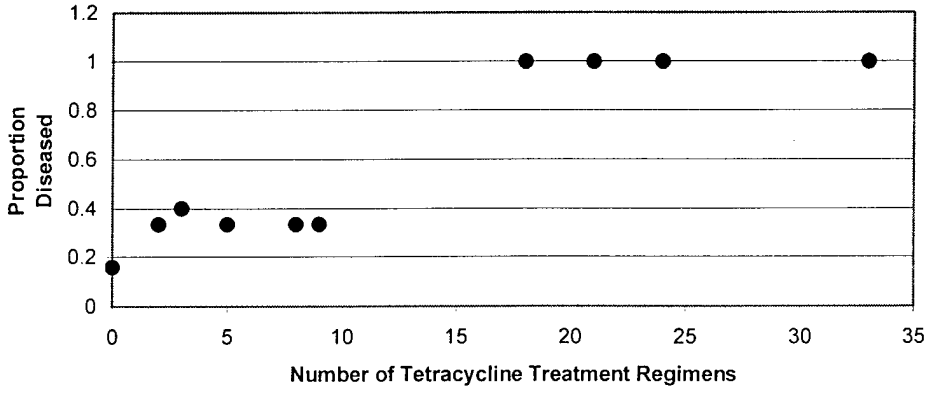


Figure 15. Plot of the proportion diseased by the number of treatment regimens of tetracycline 250 mg prescribed.

This data supports the hypothesis that a dose response relationship exists between the risk of melanoma and increasing therapies of any type of tetracycline prescribed, (i.e. the risk of melanoma increases with increasing number of treatment regimens).

Drug Specific Associations

The next research question was to see if there was a difference between the types of tetracyclines. A model was designed to evaluate the risk of melanoma with multiple categories of drug therapies. A new categorical variable was introduced based on the types of prescriptions each subject received. The five categories were defined as follows: a) 0, no tetracyclines prescribed; b) 1, only minocycline; c) 2, both of minocycline and tetracycline; d) 3, demeclocycline only; e) 4, both of tetracycline and doxycycline; f) 5, doxycycline only; and f) 5, tetracycline only.

Only tetracycline and doxycycline were prescribed in an enough amounts to test for differences. Minocycline was prescribed to 3 subjects and demeclocycline was prescribed to only to one subject.

Using a logistic regression model to compare the categories of drugs prescribed, a significant finding at the 95 % confidence level was found with the comparison of use Tetracycline only of tetracycline type drug to subjects who had no prescriptions filled of any tetracycline type of drug. The results for this model are shown in Table 8. Although no errors occurred, the model results revealed that some categories in the model were indeterminate, so the model may be considered somewhat unstable (Appendix D, Table D3). The results of this test are presented but should be interpreted with caution.

Another test was performed creating dummy variables to compare the two drugs used most, Doxycycline and Tetracycline, to those who never had a tetracycline drug prescribed. In this model, the three patients who had only minocycline or demeclocycline were not included in the analysis.

Table 8.

Multiple Risk Equation for Melanoma: Logistic Regression Model Relating Baseline Characteristics and Type of Tetracycline Prescribed to Incidence of Melanoma.

Model:

Melanoma = Only Tetracycline + Only Doxycycline + Combination of Tetracycline and Doxycycline + Only Demeclocycline + Combination of Minocycline and Tetracycline + Only Minocycline + Age + Other Photosensitive drugs+ Marital Status + Branch of Service + Combat Duty + Risk Year Group + HIV Status + Prisoner of War Status

Tetracyclines by type/use	No tetracyclines =Reference		
	Doxycycline only	1.947	1.000-3.791
	Tetracycline only	4.049	1.734-9.452
Other known photosensitizing drugs	0=not prescribed 1=prescribed	1.185	0.858-1.635
Age	1-year age difference	1.00	0.986-1.014
Marital Status	Married =Reference		
	Never Married	0.859	0.423-1.742
	Divorced	1.490	1.019-2.179
	Separated	1.574	0.613-4.038
	Widow/Widower	1.237	0.717-2.136
Prisoner of War	0=No 1=Yes	1.059	0.356-3.155
Combat Duty	0=No 1=Yes	0.951	0.688-1.314
Branch of Service	Air Force=Reference		
	US Coast Guard or Merchant Seaman	0.866	0.170-4.422
	Marine Corps	0.766	0.391-1.501
	Navy	1.101	0.680-1.783
	Army	0.683	0.452-1.032
HIV status	0=no history of seropositivity 1=seropositivity documented	1.797	0.346-9.336
Risk Year Group	1994 = Reference		
	1995	0.876	0.434-1.767
	1996	0.731	0.365-1.464
	1997	0.812	0.420-1.569
	1998	0.674	0.367-1.239
	1999	0.684	0.366-1.279
	2000	0.713	0.390-1.307

Summary of Results

Analyses of the data were performed using bivariate associations, bivariate correlations and multivariate logistic regression. Bivariate associations revealed baseline characteristics frequency distributions that were fairly comparable (Table 5). The bivariate correlations revealed no significant correlations (Appendix E, Table E1).

Multiple logistic regression revealed an unadjusted odds ratio of 2.007 with 95% confidence limits of 1.213-3.321 for those who had been prescribed any of the tetracycline type of drugs to those who had no documented prescriptions of a tetracycline drug upon entry into the study. After adjusting for eight potential risk factors, the adjusted odds ratio was 2.064, with confidence limits of 1.228-3.467.

Identification of a dose response relationship was found when the log odds for disease was plotted against the number of treatment regimens of tetracycline prescribed (Figure 13). The strength of this association was calculated using the Spearman correlation coefficient, which was .64 and found to be significant at the $p < .05$ level.

Using a logistic regression model, which included all baseline characteristics except smoking history, exposures to the different types of tetracycline drugs were categorized. When comparing subjects taking only the tetracycline type of drug versus those subjects who had no history of taking any tetracycline the risk was over four times greater for melanoma (OR=4.049, 95% confidence limits= 1.734, 9.452). Doxycycline also showed a positive association although not significant at the .05 alpha level (OR=1.947, 95% confidence level=1.000, 3.789).

Table 9 summarizes the estimated risks for melanoma for subjects exposed to tetracyclines in this study based on the analyses.

Table 9.

Summary of Findings on Risk of Melanoma With Exposure to Tetracyclines.

Exposure Category	**Odds Ratio**	**95% C.I.**
Ever vs. Never Any Type of Tetracycline	2.064	1.228 - 3.467
Tetracycline Type Only vs. Never Any Tetracycline	4.049	1.734 – 9.452
Doxycycline Only vs. Never Any Type of Tetracycline	1.947	1.000 - 3.789

Note. All Adjusted for Age, Marital Status, Branch of Service, HIV Status, POW Status, Risk Year, Combat Duty, and Other Known Photosensitizers.

CHAPTER FIVE: DISCUSSION AND CONCLUSION

Summary (Chapters 1- 3)

Supporting studies of tetracyclines as a risk factor for melanoma were not found in the literature, although melanoma researchers have speculated that drug induced photosensitization and melanoma may be associated (Stern, 1998). Flouriquinolone antibiotics are the main photosensitizing antibiotics that have been studied for their potential to increase risk for melanoma. However, these studies have been performed on animals. The animal models have shown consistently that there is increase risk for skin tumors with use of flouroquinolones (Johnson et al., 1997). Epidemiological studies involving photosensitizing drugs and subsequent development of melanoma are not abundant, and most published studies involve the association of psoralens and melanoma for those persons who have had PUVA therapy (Gupta, Stern, Swanson, & Anderson, 1988). Recent findings with patients on PUVA therapy are suggestive of a role of this type of therapy in the risk for melanoma. These few studies, and the fact that the FDA is pursuing guidance to emphasize the photosafety testing of pharmaceuticals because of the potential for photocarcinogenesis, support the hypothesis that tetracyclines may increase the risk for melanoma.

Photosensitivity reactions, which can cause clinical and subclinical cellular damage, may be a biologic reason for the increased risk. The numerous sunburn studies support an association between acute sun exposure reactions and melanoma.

With photosensitivity reactions defined as exaggerated sunburn reactions, such biological similarity should result in similar associations when tested in similar case control studies.

Discussion of Findings

The results of the analyses reveal this study population had twice the risk of melanoma (OR= 2.064, 95% C.I.= 1.228, 3.467) if they had been prescribed any type of tetracycline drug prior to diagnosis than those who did not have a prior history of any type of tetracycline prescription. Other interesting findings were the results for the individual types of tetracycline drugs. Those prescribed tetracycline, which has been on the market for 50 years, had a 4 times greater risk for melanoma (OR=4.049, 95% C.I.=1.734, 9.452) than those who had no history of any tetracycline prescription. Doxycycline showed an almost 2 times greater risk (OR=1.947, 95% C.I.=1.000, 3.789), but it has only been on the market since 1967, or for 33 years for this study population. The difference in the risks may be attributable to the length of use of the antibiotics and we may see a trend with doxycycline antibiotics within the next 10 years.

This study found a possible dose response relationship between the number of treatment regimens of any tetracycline type drug and the risk of melanoma. A linear type of relationship was noted between tetracycline type and doxycycline types of tetracycline drugs. According to Layton (Layton & Cunliffe, 1993) photosensitive reactions to tetracyclines have been reported to follow a dose response relationship.

The study's findings support a positive association between tetracyclines and melanoma. Chance could have explained these results (this case sample may have just

by chance had a higher exposure to tetracyclines), however, the statistical probability the results occurred by chance alone is 5%, based on initial alpha levels set at .05 at the onset of the study.

Strengths of the Study

The biggest strength of this study is its biologic plausibility. Almost all researchers of melanoma agree with a sun exposure relationship. The sun damages the cell and causes DNA changes with resulting melanoma. Photosensitizing drugs intensify this damage and therefore should result in more rapid development of melanoma.

The design of the study also has its strengths. Random selection of controls from the same source population as the cases helped minimize selection biases and assured a reasonably representative sample. Selection on age and gender ensured the range of ages and gender was similar to the cases. Selection of controls from patients at risk of diagnosis (seen as a patient the same year as case diagnosed) ensured secular time biases were reduced. This design also gave the advantage of having the exposures already identified before case selection, so no recall or researcher biases occurred.

Weaknesses of the Study

Reliability and Validity

Overview

Secondary data analyses have inherent weaknesses due to the dependence on data availability. Thus, one of the single greatest weaknesses in studies utilizing computerized databases may deal with the reliability and the validity of the data.

Kashner (1998) found, in a study of a national random sample of VA outpatient and inpatient visits, that agreement was adequate for demographics (e.g. gender, date of birth, branch of service, etc) with a kappa of .92, and selected diagnoses (kappa = .39 to 1.0) when compared to patient medical charts in the VA health information system. Most of the variables came from the demographic database, and, thus, the information is probably considered a valid measurement.

In this study, missing data severely limited the analyses and interpretation of the results. Missing entries in databases cannot automatically be assumed to occur at random. There were over 6000 gender fields missing in the patient visit records and almost 800 ethnicities missing from the original selected cases and controls. It was thought that the ethnicity data was missing due to changes in patient sensitivity policies. This may have occurred over a specific time frame with some of the subjects systematically excluded. Older providers may not be as sensitive and write ethnicity in their notes while younger providers might not reference ethnicity.

In the original population selected (N=2167), a comparison of those with missing ethnicity data to those with documented ethnicity revealed a two fold greater risk of melanoma (OR= 2.171, 95% C.I.=1.552, 3.036) if the ethnicity was unknown, after adjusting for all baseline characteristics. However, after adjusting for unknown ethnicity, the risk for exposure to tetracycline type drugs was still one and a half times the risk of those who were not prescribed tetracyclines (OR=1.578, 95% C.I.= 0.997, 2.498).

Biases

Although the random selection of controls minimized the selection bias in this study other biases could have occurred that may have affected the association.

Selection biases. The sensitivity of capturing the disease in these patients may be higher than in the controls. Doctors may see patients who require tetracycline antibiotics more, increasing the surveillance for the disease. Patients with melanoma may get prescribed tetracyclines more often for some premelanoma skin condition. A cursory review of 15 subjects' computerized records, which were prescribed the greatest number of regimens of tetracycline, revealed no trend on physician or clinic, which prescribed the drugs the most. Clinics included podiatry, ophthalmology, urology, gastroenterology, dental, and dermatology. Diagnoses included nasal obstruction, ingrown toenail, keratosis and non-specific dermatitis, lung cancer, bronchitis, foot ulcer, prostate cancer, lacrimal duct infection, esophagitis, and nonspecified dental diagnosis.

Misclassification of exposure. There can definitely be some misclassification of exposure. It is not known if the patient actually took the pills, it is just documented that they picked them up from the pharmacy. Patients may have unrecorded prescriptions that they received from outside the VA pharmacy. They may be taking other drugs or supplements that also cause photosensitization reactions. Biological differences in reactions to medications (either clinical or subclinical) can bias the results. Exposure time may be questionable because exposure only had to be one year prior to entry into the study. All information for exposure was dependent on physical entry into computer

and the person entering the data. All of these may result in differential or nondifferential biases.

Misclassification of disease. Misclassification of disease could have occurred in the study. The agreement of pathologists on diagnosing melanoma is not 100% (Heenan et al., 1984), so some patients will get diagnosed with the disease that don't have it and others with the disease may not be diagnosed. This may also result in differential or nondifferential bias.

Any of these biases could have affected the tetracycline melanoma association. However, with the random selection of controls, the use of all cases from the source population, and use of exposures known prior to diagnosis, most of these biases would be expected to be nondifferential, and, thus, have caused the association to be closer to the null.

Generalizability

The final study population only included White males, and this may affect generalizability. However, they should be representative of white males throughout the United States, if 1 in 4 adult males or 25% of the male population in the U.S. truly are veterans.

Uncontrolled Confounding

Secondary data analyses severely limit the information available to the researcher. Many suspected confounders go uncontrolled because of this lack of information. Although in this study the tetracycline melanoma association remained after adjusting for some potential risk factors, many other suspected confounders were not controlled for such as sun exposure and familial histories. The major explanation

for this association if a true relationship does not exist may be uncontrolled confounding. Because exposure to sun and familial history along with prior diagnoses of another skin cancer are such strong risk factors for this disease, adjustment with these factors may remove the association. Only with adjustment of some of these other strong confounders can the association clearly be defined. However, if physicians are instructing their patients using tetracyclines to stay out of the sun, then this should reduce sun exposure and the influence it has on the tetracycline melanoma association.

Implications and Future Directions

Although the study has many limitations, the findings of a significant greater risk associated with exposure to any tetracycline type drug; the positive associations found with tetracycline, doxycycline, and other photosensitizing drugs; along with a dose response relationship, support the hypothesis that tetracyclines are associated with increased risk of melanoma.

With the melanoma rate in the male veterans at this hospital 3 to 4 times higher in men older than 55 than those reported in the SEER males, and the annual prescription rate double the national rates reported on Johns Hopkins' Infectious Disease Division website, this population is an ideal population for further research into the association of melanoma and tetracyclines. Greater than 90% of the cases in this study were born before 1951, when tetracyclines were the number one prescribed broad-spectrum antibiotic in the United States. At that time, the phototoxic effects of the tetracyclines were not known, and no warnings to stay out of the sun were issued by their prescribers. Their potential for previous photosensitive reactions is great.

Clinically, these results should alert physicians and patients who have other risk factors for melanoma. Awareness of prior use of tetracyclines, such as stained teeth or history of rash or hyperpigmentation reactions, should clue a provider and researcher in on possible exposures and recognize their patient may have an increased risk than those that do not. Patients who have clinical photosensitizing reactions should be included in prospective studies for melanoma research. Future studies into the risk of melanoma and tetracyclines should also include longer drug histories prior to diagnosis, prior histories of other skin cancers or skin disease, sun exposure and familial histories. A new study using this population could be conducted to answer these questions. An analogous study in the active duty population could be conducted to see if similar results are obtained.

As risk factors for melanoma are identified, it is apparent as in any disease, that in any one person, one risk factor may be more prominent than others (American Academy of Dermatology, 2000). In another person, no one risk factor may be apparent. Some investigators believe that, in most people, the risk factors for melanoma have a complex inter-relationship that makes it difficult to identify any single factor as the "cause" of melanoma in any individual. This study was not designed to prove causality of a tetracycline melanoma association but to explore a new potential risk factor for melanoma in a study population with a history of high rates of skin cancer. Knowledge of an increase risk with use of tetracycline drugs can aid in more prudent use for nonessential therapy.

This study's results warrant the continued monitoring of patients on tetracyclines for risk of melanoma and, if future studies are consistent with these findings, labels should include warnings of these risks.

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APPENDICES

Appendix A: Proposed Staging Criteria

Table A1.
Revised TNM Staging for Malignant Melanoma that will be adopted in 2002

Tumor Stage	Tumor Thickness
T1	</= 1.0 mm
T2	1.01 mm to 2.0 mm
T3	2.01 mm to 4.0 mm
T4	> 4.0 mm

T(a) in T1 disease denotes lesions without ulceration and level 2/3.

T(b) in T1 disease denotes lesions with ulceration OR level 4/5 disease.

For all other T categories, (a) means without ulceration, (b) means with ulceration.

N (node)	Involvement
N1	1 node
N2	2 to 3 nodes
N3	>/= 4 nodes OR matted nodes OR in-transit/satellite metastases and nodal metastases

N(a) denotes micrometastasis.

N(b) denotes macrometastasis.

N(c) used in N2 subclassification to describe the presence of in-transit/ satellite metastasis without nodal metastasis.

M	(metastasis)
M1a	distant skin, subcutaneous or nodal metastases with normal LDH
M1b	lung metastases with normal LDH
M1c	all other visceral metastases with normal LDH or any distant metastases with elevated LDH

Note. LDH=lactate dehydrogenase. Both clinical and pathologic staging are included. the latter determined either by selective or complete lymphadenectomy.

Appendix B: Tetracycline Uses and Adverse Affects

Table B1.

Current uses of the tetracycline class of drugs.

Labeled Uses	Unlabeled Uses (not currently included in labeling approved by the FDA.)
Acne Vulgaris	Bejel
Actinomycosis	Biliary Tract Infection
Anthrax	Chlamydial Infections
Bronchitis	Extraintestinal Amebiasis
Brucellosis	Intra-Abdominal Infection
Gen-Urin. Chlamydia Trachomatis	Malaria, P. Falciparum, Chloroquine Resist
Gingivostomatitis	Malaria, Unspecified
Gonococcal Cervicitis, Acute	Ocular Rosacea
Gonococcal Endometritis, Acute	Peptic Ulcer Due To H. Pylori
Gonococcal Epididymo-Orchitis, Acute	Pinta
Gonococcal Pharyngitis	Plague
Gonorrhea, Disseminated	Pneumonia, Mycoplasmal
Gonorrhea, Lower Genitourinary, Acute	Rosacea, Acne
Gonorrhea, Rectal	Septicemia
Gonorrhea, Urethritis Acute	Tularemia
Granuloma Inguinale	
Inclusion Conjunctivitis	
Lymphogranuloma Venereum	
Otitis Media Infection	
Pharyngitis	
Pneumonia	
Psittacosis	
Q Fever	
Rectal Inf.Chlamydia Trachomatis	
Relapsing Fever	
Rickettsial Pox	
Rocky Mountain Spotted Fever	
Sinusitis	
Skin And Soft Tissue Infections	
Syphilis	
Trachoma	
Typhus Infections	
Urinary Tract Infections	
Yaws	

Table B2.

Adverse reactions associated with the class of tetracyclines.

Gastrointestinal	Anorexia, epigastric distress, nausea, vomiting, diarrhea, bulky loose stools, stomatitis, sore throat, glossitis, black hairy tongue, dysphagia, hoarseness, enterocolitis, and inflammatory lesions (with monilial overgrowth) in the anogenital region, including proctitus and pruritus ani. These reactions have been caused by both the oral and parenteral administration of tetracyclines.
Skin	Photosensitivity. Maculopapular and erythematous rashes. Exfoliative dermatitis has been reported but is uncommon.
Renal toxicity	Rise in BUN has been reported and is apparently dose related.
Liver	Hepatic cholestasis has been reported rarely, and is usually associated with high dosage levels of tetracycline.
Hypersensitivity reactions	Urticaria, angioneurotic edema, anaphylaxis, anaphylactoid purpura, pericarditis and exacerbation of systemic lupus erythematosus, and serum sickness-like reactions, as fever, rash, and arthralgia. Bulging fontanels have been reported in young infants following full therapeutic dosage. This sign disappeared rapidly when the drug was discontinued.
Blood	Anemia, hemolytic anemia, thrombocytopenia, thrombocytopenic purpura, neutropenia and eosinophilia have been reported.
CNS	Dizziness / light headedness is commonly seen with minocycline, but not the others. This is caused by vestibular or CNS toxicity and is of such severity and frequency that CDC has changed recommendations on its non-essential use.
Thyroid	When given over prolonged periods, tetracyclines have been reported to produce brown-black microscopic discoloration of thyroid glands. No abnormalities of thyroid function studies are known to occur.
Bone, Teeth	Deposition in calcified tissues, e.g., teeth can result in discoloration, especially when given during developmental stages. Higher doses given at inopportune stages of growth can result in bone deformation. Nearly everyone who received tetracyclines as a child will have teeth that fluoresce under a UV light source whether their teeth are stained brown or not.

Appendix C: Selected Known Photosensitizers

Table C1.
Other Selected Known Photosensitizing Drugs from the J.A. Haley Pharmacy Database.

Amantadine HCL 100mg Cap	Diltiazem (Cardizem Sr) 120mg SA Cap
Amiodarone HCL 200mg Tab	Diltiazem (Cardizem Sr) 120mg SA Cap Ud
Chloroquine Phosphate 500mg Tab	Diltiazem (Dilacor Xr) 120mg Sa Cap
Alprazolam 0.25mg Tab	Diltiazem (Tiazac) 120mg Sa Cap
Alprazolam 0.5mg Tab	Diltiazem (Tiazac) 180mg Sa Cap
Alprazolam 1mg Tab	Diltiazem (Tiazac) 180mg Sa Cap Ud
Ciprofloxacin 500mg Tab Ud	Diltiazem (Tiazac) 240mg Sa Cap
Ciprofloxacin HCL 0.3% Oph Soln	Diltiazem (Tiazac) 300mg Sa Cap
Ciprofloxacin HCL 500mg Tab	Diltiazem (Tiazac) 360mg Sa Cap
Ciprofloxacin HCL 750mg Tab	Diltiazem (Tiazac) 360mg Sa Cap Ud
Amantadine HCL 100mg Cap	Diltiazem 180mg Sa Cap
Coal Tar 1% Shampoo 240ml	Diltiazem Cd 180 Mg Cap Ud
Coal Tar 2.5% W/Lanolin,	Diltiazem Cd 180 Mg Cap Ud
240ml Bath Oil	Diltiazem HCL 30mg Tab
Coal Tar 5% Top Gel	Diltiazem HCL 60mg Sa Cap
Coal Tar 5%/2% Sulfur &	Diltiazem HCL 60mg Tab
Sal Acid Shampoo	Diltiazem HCL 90mg Sa Cap
Griseofulvin Microsize 250mg Tab	Methoxsalen (8-Mop) 10mg Cap
Griseofulvin Microsize 500mg Tab	Ofloxacin 0.3% Oph Soln
Auranofin 3mg Cap	Ofloxacin 200mg Tab
Chlorpromazine 25mg Rtl Supp	Ofloxacin 400mg Tab
Chlorpromazine HCL 100mg Tab	Pyrazinamide 500mg Tab
Chlorpromazine HCL 10mg Tab	Etretinate 25mg Cap

Note: These drugs were included in the variable used in the analyses defined as "Other Known Photosensitizing Drugs".

Appendix D: Results from SAS Analyses

The CORR Procedure

8 Variables: tottet marital pow1 combat1 hiv1 group force photo

Simple Statistics

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum	Label
tottet	1271	0.06688	0.24991	85.00000	0	1.00000	
marital	1271	4.08655	1.39020	5194	1.00000	5.00000	
marital							
pow1	1271	0.02282	0.14938	29.00000	0	1.00000	pow1
combat1	1271	0.36664	0.48208	466.00000	0	1.00000	
combat1							
hiv1	1271	0.00708	0.08388	9.00000	0	1.00000	hiv1
group	1271	1998	1.86923	2539104	1994	2000	group
force	1266	3.80569	0.81981	4818	1.00000	5.00000	force
photo	1271	0.32494	0.46854	413.00000	0	1.00000	

Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	tottet	marital	pow1	combat1	hiv1	group	force	photo
tottet	1.00000	0.03092	-0.01981	0.00546	0.01495	0.04665	-0.00571	0.13705
		0.2706	0.4803	0.8458	0.5943	0.0964	0.8391	<.0001
	1271	1271	1271	1271	1271	1271	1266	1271
marital	0.03092	1.00000	0.07769	0.07128	-0.03902	0.01868	0.01275	0.04746
marital	0.2706		0.0056	0.0110	0.1644	0.5059	0.6504	0.0908
	1271	1271	1271	1271	1271	1271	1266	1271
pow1	-0.01981	0.07769	1.00000	-0.12430	-0.01290	-0.00542	0.06208	-0.02726
pow1	0.4803	0.0056		<.0001	0.6458	0.8469	0.0272	0.3315
	1271	1271	1271	1271	1271	1271	1266	1271
combat1	0.00546	0.07128	0.12430	1.00000	0.01363	-0.00193	-0.07884	0.01596
combat1	0.8458	0.0110	<.0001		0.6272	0.9452	0.0050	0.5698
	1271	1271	1271	1271	1271	1271	1266	1271
hiv1	0.01495	-0.03902	-0.01290	0.01363	1.00000	-0.01252	-0.02583	-0.03856
hiv1	0.5943	0.1644	0.6458	0.6272		0.6556	0.3585	0.1695
	1271	1271	1271	1271	1271	1271	1266	1271
group	0.04665	0.01868	-0.00542	-0.00193	-0.01252	1.00000	0.03721	-0.01975
group	0.0964	0.5059	0.8469	0.9452	0.6556		0.1858	0.4817
	1271	1271	1271	1271	1271	1271	1266	1271
force	-0.00571	0.01275	0.06208	-0.07884	-0.02583	0.03721	1.00000	-0.00771
force	0.8391	0.6504	0.0272	0.0050	0.3585	0.1858		0.7841
	1266	1266	1266	1266	1266	1266	1266	1266
photo	0.13705	0.04746	-0.02726	0.01596	-0.03856	-0.01975	-0.00771	1.00000
	<.0001	0.0908	0.3315	0.5698	0.1695	0.4817	0.7841	
	1271	1271	1271	1271	1271	1271	1266	1271

Figure D1.
Correlation matrix for all variables.

The LOGISTIC Procedure

Model Information

Data Set	WORK.SMOKE
Response Variable	disease
Number of Response Levels	2
Number of Observations	1266
Link Function	Logit
Optimization Technique	Fisher's scoring

Response Profile

Ordered Value	disease	Total Frequency
1	1	207
2	0	1059

NOTE: 5 observations were deleted due to missing values for the response or explanatory variables.

Class Level Information

		Design Variables					
Class	Value	1	2	3	4	5	6
force	1	1	0	0	0		
	2	0	1	0	0		
	3	0	0	1	0		
	4	0	0	0	1		
	5	-1	-1	-1	-1		
marital	1	1	0	0	0		
	2	0	1	0	0		
	3	0	0	1	0		
	4	0	0	0	1		
	5	-1	-1	-1	-1		
group	1994	-1	-1	-1	-1	-1	-1
	1995	1	0	0	0	0	0
	1996	0	1	0	0	0	0
	1997	0	0	1	0	0	0
	1998	0	0	0	1	0	0
	1999	0	0	0	0	1	0
	2000	0	0	0	0	0	1
photo	0	-1					
	1	1					

Model Convergence Status

Convergence criterion (GCONV=1E-8) satisfied.

Figure D2.
Full Model Results from SAS using Ever/Never Tetracycline Variable.

Figure D2. (continued)

Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	1129.854	1146.320
SC	1134.998	1254.336
-2 Log L	1127.854	1104.320

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	23.5339	20	0.2633

Type III Analysis of Effects

Effect	DF	WaldChi-Square	Pr > ChiSq
tottet	1	7.4873	0.0062
photo	1	0.9137	0.3391
AGE	1	0.0007	0.9783
marital	4	5.5563	0.2348
force	4	6.8135	0.1461
combat1	1	0.0544	0.8155
group	6	2.7008	0.8454
hiv1	1	0.2243	0.6358
pow1	1	0.0028	0.9579

Analysis of Maximum Likelihood Estimates

Parameter		DF	Estimate	Standard Error	Chi-Square	Pr > ChiSq
Intercept		1	-1.4250	0.5155	7.6424	0.0057
tottet		1	0.7244	0.2647	7.4873	0.0062
photo	1	1	0.0783	0.0819	0.9137	0.3391
AGE		1	-0.00019	0.00698	0.0007	0.9783
marital	1	1	-0.3266	0.2978	1.2023	0.2729
marital	2	1	0.2103	0.1844	1.3010	0.2540
marital	3	1	0.2623	0.3871	0.4591	0.4981
marital	4	1	0.0420	0.2410	0.0304	0.8616
force	1	1	0.0846	0.6453	0.0172	0.8957
force	2	1	-0.1431	0.2864	0.2499	0.6172
force	3	1	0.1805	0.2199	0.6738	0.4117
force	4	1	-0.2614	0.1967	1.7654	0.1839
combat1		1	-0.0384	0.1644	0.0544	0.8155
group	1995	1	0.1233	0.2238	0.3033	0.5818
group	1996	1	-0.0423	0.2197	0.0371	0.8473
group	1997	1	0.0440	0.1989	0.0488	0.8251
group	1998	1	-0.1193	0.1667	0.5115	0.4745
group	1999	1	-0.1446	0.1778	0.6618	0.4159
group	2000	1	-0.1204	0.1651	0.5314	0.4660
hiv1		1	0.3896	0.8226	0.2243	0.6358
pow1		1	-0.0293	0.5555	0.0028	0.9579

Figure D2. (*continued*)

Odds Ratio Estimates

Effect		Point Estimate	95% Wald Confidence Limits	
tottet		2.064	1.228	3.467
photo	1 vs 0	1.169	0.848	1.612
AGE		1.000	0.986	1.014
marital	1 vs 5	0.871	0.431	1.761
marital	2 vs 5	1.489	1.020	2.174
marital	3 vs 5	1.569	0.611	4.025
marital	4 vs 5	1.259	0.733	2.161
force	1 vs 5	0.947	0.190	4.724
force	2 vs 5	0.754	0.387	1.470
force	3 vs 5	1.042	0.646	1.681
force	4 vs 5	0.670	0.444	1.010
combat1		0.962	0.697	1.328
group	1995 vs 1994	0.873	0.433	1.758
group	1996 vs 1994	0.740	0.370	1.477
group	1997 vs 1994	0.806	0.418	1.555
group	1998 vs 1994	0.685	0.374	1.256
group	1999 vs 1994	0.668	0.358	1.246
group	2000 vs 1994	0.684	0.374	1.251
hiv1		1.476	0.294	7.404
pow1		0.971	0.327	2.885

Association of Predicted Probabilities and Observed Responses

Percent Concordant	59.1	Somers' D	0.203
Percent Discordant	38.8	Gamma	0.208
Percent Tied	2.1	Tau-a	0.056
Pairs	219213	c	0.602

The LOGISTIC Procedure

Model Information

Data Set	WORK.TWO	
Response Variable	disease	disease
Number of Response Levels	2	
Number of Observations	1263	
Link Function	Logit	
Optimization Technique	Fisher's scoring	

Response Profile

Ordered Value	disease	Total Frequency
1	1	207
2	0	1056

NOTE: 5 observations were deleted due to missing values for the response or explanatory variables.

Class Level Information

		Design Variables					
Class	Value	1	2	3	4	5	6
force	1	1	0	0	0		
	2	0	1	0	0		
	3	0	0	1	0		
	4	0	0	0	1		
	5	-1	-1	-1	-1		
marital	1	1	0	0	0		
	2	0	1	0	0		
	3	0	0	1	0		
	4	0	0	0	1		
	5	-1	-1	-1	-1		
group	1994	1	0	0	0	0	0
	1995	0	1	0	0	0	0
	1996	0	0	1	0	0	0
	1997	0	0	0	1	0	0
	1998	0	0	0	0	1	0
	1999	0	0	0	0	0	1
	2000	-1	-1	-1	-1	-1	-1
photo	0	1					
	1	-1					

Model Convergence Status

Convergence criterion (GCONV=1E-8) satisfied.

Figure D3.
Full Model Using SAS With Drug Specific Dummy Variables For Tetracycline Exposure.

Figure D3. *(continued)*

Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	1128.781	1140.814
SC	1133.923	1259.063
-2 Log L	1126.781	1094.814

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	31.9674	22	0.0780
Score	34.0867	22	0.0481
Wald	30.2343	22	0.1129

Type III Analysis of Effects

Effect	DF	Wald Chi-Square	Pr > ChiSq
tet	1	10.4533	0.0012
dox	1	3.8373	0.0501
both	1	0.0004	0.9835
AGE	1	0.0000	0.9988
marital	4	5.5856	0.2323
combat1	1	0.1147	0.7349
group	6	2.5445	0.8635
force	4	7.2995	0.1209
photo	1	1.0146	0.3138
hiv1	1	0.4858	0.4858
pow1	1	0.0120	0.9129

Analysis of Maximum Likelihood Estimates

Parameter		DF	Estimate	Standard Error	Chi-Square	Pr > ChiSq
Intercept		1	-1.4575	0.5206	7.8390	0.0051
tet		1	1.3985	0.4325	10.4533	0.0012
dox		1	0.6661	0.3400	3.8373	0.0501
both		1	-13.3509	644.9	0.0004	0.9835
AGE		1	-0.00001	0.00703	0.0000	0.9988
marital	1	1	-0.3335	0.2989	1.2449	0.2645
marital	2	1	0.2172	0.1850	1.3779	0.2405
marital	3	1	0.2680	0.3873	0.4790	0.4889
marital	4	1	0.0327	0.2428	0.0181	0.8930
combat1		1	-0.0559	0.1651	0.1147	0.7349
group	1994	1	0.2524	0.2327	1.1758	0.2782
group	1995	1	0.1203	0.2244	0.2874	0.5919
group	1996	1	-0.0581	0.2206	0.0693	0.7924
group	1997	1	0.0446	0.1998	0.0499	0.8233
group	1998	1	-0.1498	0.1684	0.7921	0.3735
group	1999	1	-0.1248	0.1786	0.4880	0.4848
force	1	1	-0.00032	0.6536	0.0000	0.9996
force	2	1	-0.1249	0.2889	0.1868	0.6656
force	3	1	0.2333	0.2224	1.1006	0.2941
force	4	1	-0.2472	0.1985	1.5497	0.2132
photo	0	1	-0.0828	0.0822	1.0146	0.3138
hiv1		1	0.5860	0.8409	0.4858	0.4858
pow1		1	0.0609	0.5569	0.0120	0.9129

Figure D3. *(Continued)*

Odds Ratio Estimates

Effect		Point Estimate	95% Wald Confidence Limits	
tet		4.049	1.734	9.452
dox		1.947	1.000	3.791
both		<0.001	<0.001	>999.999
AGE		1.000	0.986	1.014
marital	1 vs 5	0.862	0.425	1.747
marital	2 vs 5	1.494	1.022	2.184
marital	3 vs 5	1.572	0.613	4.035
marital	4 vs 5	1.242	0.720	2.143
combat1		0.946	0.684	1.307
group	1994 vs 2000	1.401	0.765	2.565
group	1995 vs 2000	1.227	0.682	2.210
group	1996 vs 2000	1.027	0.575	1.834
group	1997 vs 2000	1.138	0.663	1.954
group	1998 vs 2000	0.937	0.580	1.513
group	1999 vs 2000	0.961	0.584	1.580
force	1 vs 5	0.870	0.171	4.428
force	2 vs 5	0.768	0.392	1.504
force	3 vs 5	1.099	0.679	1.779
force	4 vs 5	0.680	0.450	1.027
photo	0 vs 1	0.847	0.614	1.170
hiv1		1.797	0.346	9.338
pow1		1.063	0.357	3.166

Association of Predicted Probabilities and Observed Responses

Percent Concordant	59.9	Somers' D	0.215
Percent Discordant	38.4	Gamma	0.218
Percent Tied	1.7	Tau-a	0.059
Pairs	218592	c	0.607

Model Information

Data Set	WORK.BLAIR2
Response Variable	disease
Number of Response Levels	2
Number of Observations	1266
Link Function	Logit
Optimization Technique	Fisher's scoring

Response Profile

Ordered Value	disease	Total Frequency
1	1	207
2	0	1059

NOTE: 5 observations were deleted due to missing values for the response or explanatory variables.

Class Level Information

		Design Variables					
Class	Value	1	2	3	4	5	6
force	1	1	0	0	0		
	2	0	1	0	0		
	3	0	0	1	0		
	4	0	0	0	1		
	5	-1	-1	-1	-1		
marital	1	1	0	0	0		
	2	0	1	0	0		
	3	0	0	1	0		
	4	0	0	0	1		
	5	-1	-1	-1	-1		
group	1994	-1	-1	-1	-1	-1	-1
	1995	1	0	0	0	0	0
	1996	0	1	0	0	0	0
	1997	0	0	1	0	0	0
	1998	0	0	0	1	0	0
	1999	0	0	0	0	1	0
	2000	0	0	0	0	0	1
photo	0	-1					
	1	1					
combat1	0	-1					
	1	1					
hiv1	0	-1					
	1	1					
pow1	0	-1					
	1	1					

Model Convergence Status

Convergence criterion (GCONV=1E-8) satisfied.

Figure D4.
Full Model Using Individual Variables for Drug Specific Exposures.

Figure D4. *(continued)*

Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
-2 Log L	1127.854	1093.921

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	33.9326	25	0.1094

Type III Analysis of Effects

Effect	DF	Wald Chi-Square	Pr > ChiSq
tet	1	11.0981	0.0009
dox1	1	3.8346	0.0502
tetdox	1	0.0004	0.9835
dem1	1	0.0001	0.9939
mintet	1	0.0001	0.9941
min1	1	0.0001	0.9913
AGE	1	0.0001	0.9944
photo	1	1.0625	0.3026
marital	4	5.5305	0.2371
force	4	7.1820	0.1266
combat1	1	0.0928	0.7606
group	6	2.5010	0.8684
hiv1	1	0.4858	0.4858
pow1	1	0.0107	0.9176

Analysis of Maximum Likelihood Estimates

Parameter		DF	Estimate	Standard Error	Chi-Square	Pr > ChiSq
Intercept		1	-1.1634	0.6831	2.9009	0.0885
tet		1	1.4608	0.4385	11.0981	0.0009
dox1		1	0.6658	0.3400	3.8346	0.0502
tetdox		1	-13.3512	645.8	0.0004	0.9835
dem1		1	-13.3546	1736.4	0.0001	0.9939
mintet		1	-12.9228	1736.4	0.0001	0.9941
min1		1	-13.3512	1225.2	0.0001	0.9913
AGE		1	-0.00005	0.00702	0.0001	0.9944
photo	1	1	0.0847	0.0822	1.0625	0.3026
marital	1	1	-0.3348	0.2990	1.2542	0.2628
marital	2	1	0.2163	0.1851	1.3654	0.2426
marital	3	1	0.2708	0.3873	0.4887	0.4845
marital	4	1	0.0305	0.2430	0.0157	0.9003
force	1	1	-0.00454	0.6548	0.0000	0.9945
force	2	1	-0.1276	0.2892	0.1946	0.6591
force	3	1	0.2352	0.2227	1.1157	0.2909
force	4	1	-0.2422	0.1989	1.4828	0.2233
combat1	1	1	-0.0252	0.0826	0.0928	0.7606
group	1995	1	0.1197	0.2244	0.2847	0.5937
group	1996	1	-0.0608	0.2207	0.0759	0.7829
group	1997	1	0.0440	0.1999	0.0484	0.8258
group	1998	1	-0.1425	0.1683	0.7171	0.3971
group	1999	1	-0.1271	0.1787	0.5059	0.4769
group	2000	1	-0.0854	0.1658	0.2656	0.6063
hiv1	1	1	0.2930	0.4204	0.4858	0.4858
pow1	1	1	0.0288	0.2785	0.0107	0.9176

Figure D4. *(Continued)*

Odds Ratio Estimates

Effect			Point Estimate	95% Wald Confidence Limits	
tet			4.309	1.825	10.178
dox1			1.946	0.999	3.789
tetdox			<0.001	<0.001	>999.999
dem1			<0.001	<0.001	>999.999
mintet			<0.001	<0.001	>999.999
min1			<0.001	<0.001	>999.999
AGE			1.000	0.986	1.014
photo	1 vs 0		1.185	0.858	1.635
marital	1 vs 5		0.859	0.423	1.742
marital	2 vs 5		1.490	1.019	2.179
marital	3 vs 5		1.574	0.613	4.038
marital	4 vs 5		1.237	0.717	2.136
force	1 vs 5		0.866	0.170	4.422
force	2 vs 5		0.766	0.391	1.501
force	3 vs 5		1.101	0.680	1.783
force	4 vs 5		0.683	0.452	1.032
combat1	1 vs 0		0.951	0.688	1.314
group	1995	vs 1994	0.876	0.434	1.767
group	1996	vs 1994	0.731	0.365	1.464
group	1997	vs 1994	0.812	0.420	1.569
group	1998	vs 1994	0.674	0.367	1.239
group	1999	vs 1994	0.684	0.366	1.279
group	2000	vs 1994	0.713	0.390	1.307
hiv1	1 vs 0		1.797	0.346	9.336
pow1	1 vs 0		1.059	0.356	3.155

Association of Predicted Probabilities and Observed Responses

Percent Concordant	60.1	Somers' D	0.218
Percent Discordant	38.3	Gamma	0.222
Percent Tied	1.7	Tau-a	0.060
Pairs	219213	c	0.609

Total Tetracycline variable of Therapy Regimen

The LOGISTIC Procedure

Model Information

Data Set	A.SMOKE	
Response Variable	disease	disease
Number of Response Levels	2	
Number of Observations	1271	
Link Function	Logit	
Optimization Technique	Fisher's scoring	

Response Profile

Ordered Value	disease	Total Frequency
1	1	208
2	0	1063

Model Convergence Status

Convergence criterion (GCONV=1E-8) satisfied.

Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	1134.904	1134.396
SC	1140.051	1144.691
-2 Log L	1132.904	1130.396

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	2.5078	1	0.1133
Score	3.1252	1	0.0771
Wald	2.6807	1	0.1016

Figure D5.
Calculated crude odds ratios using SAS logistic regression.

Figure D5. (*continued*)

The LOGISTIC Procedure

Analysis of Maximum Likelihood Estimates

Parameter	DF	Estimate	Standard Error	Chi-Square	Pr > ChiSq
Intercept	1	-1.6512	0.0771	458.1570	<.0001
totdose	1	0.0245	0.0150	2.6807	0.1016

Odds Ratio Estimates

Effect	Point Estimate	95% Wald Confidence Limits	
totdose	1.025	0.995	1.055

Association of Predicted Probabilities and Observed Responses

Percent Concordant	10.8	Somers' D	0.053
Percent Discordant	5.5	Gamma	0.327
Percent Tied	83.8	Tau-a	0.015
Pairs	221104	c	0.527

Adjusted Odds Ratios

Effect	Unit	Estimate
totdose	5.0000	1.130

Total Tetracycline variable of Yes No variable

The LOGISTIC Procedure

Model Information

Data Set	A.SMOKE	
Response Variable	disease	disease
Number of Response Levels	2	
Number of Observations	1271	
Link Function	Logit	
Optimization Technique	Fisher's scoring	

Response Profile

Ordered Value	disease	Total Frequency
1	1	208
2	0	1063

Model Convergence Status

Convergence criterion (GCONV=1E-8) satisfied.

Model Fit Statistics

Criterion	Intercept Only	Intercept and Covariates
AIC	1134.904	1130.221
SC	1140.051	1140.516
-2 Log L	1132.904	1126.221

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	6.6831	1	0.0097
Score	7.6109	1	0.0058
Wald	7.3544	1	0.0067

Figure D6.
SAS output from crude model logistic regression analysis for yes no tetracycline ever prescribed.

The LOGISTIC Procedure

Analysis of Maximum Likelihood Estimates

Parameter	DF	Estimate	Standard Error	Chi-Square	Pr > ChiSq
Intercept	1	-1.6884	0.0800	445.1140	<.0001
tottet	1	0.6968	0.2569	7.3544	0.0067

Odds Ratio Estimates

Effect	Point Estimate	95% Wald Confidence Limits	
tottet	2.007	1.213	3.321

Association of Predicted Probabilities and Observed Responses

Percent Concordant	10.4	Somers' D	0.052
Percent Discordant	5.2	Gamma	0.335
Percent Tied	84.4	Tau-a	0.014
Pairs	221104	c	0.526

Figure D7.
SAS output from crude model logistic regression analysis for one regimen of any tetracycline prescribed.

About the Author

Nancy K. Fagan received a Doctor of Veterinary Medicine Degree from Louisiana State University in 1988. After graduation she was a mixed animal practitioner in Bradenton, Florida. In 1990, she was commissioned as a Captain in the United States Air Force and stationed at Columbus Air Force Base (AFB), Mississippi as the base Public Health Officer. In 1994 to 1996 she was transferred to Andersen AFB, Guam, and then Hickam AFB, Hawaii, until 1998. She served as Deputy Public Health Commander for the Pacific Air Forces and directed the public health of a 6000+ Kurdish refugee population. In 1998, she was selected as the Air Force's Outstanding Public Health Officer of the Year, was promoted to the rank of Major, and began the Ph.D. program at the University of South Florida in the fall. She has coauthored a publication in the American Veterinary Medical Association Journal of Research.

End